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Rudolph J. Pretorius

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THE EFFECT OF AGRICULTURAL PRACTICES ON SUGAR BEET ROOT APHID  
(*PEMPHIGUS BETAE* DOANE) AND BENEFICIAL EPIGEAL ARTHROPODS

by

Rudolph J. Pretorius

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Entomology

Under the Supervision of Professors J.D. Bradshaw & G.L. Hein

Lincoln, Nebraska

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THE EFFECT OF AGRICULTURAL PRACTICES ON SUGAR BEET ROOT APHID  
(*PEMPHIGUS BETAE* DOANE) AND BENEFICIAL EPIGEAL ARTHROPODS

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University of Nebraska, 2014

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This study investigated the effect of several agricultural practices on the sugar beet root aphid (*Pemphigus betae* Doane) and beneficial epigeal natural enemies in western Nebraska sugar beet agroecosystems. Eight glyphosate-tolerant sugar beet varieties were evaluated under field conditions for their resistance to root aphids. High levels of aphid resistance were detected for some varieties. In conjunction to this, pitfall sampling was conducted to determine the beneficial epigeal natural enemy complex in the area, which could contribute to the management of both root aphids and glyphosate-resistant weeds. Ground beetles comprised an important and abundant component of this fauna, with 79 species collected throughout this study.

The second investigated the impact of a seed-applied insecticide, Poncho Beta (clothianidin and beta-cyfluthrin), and sugar beet plant density (25,000 plants/acre vs. 35,000 plants/acre) on root aphids and beneficial epigeal arthropods. The insecticide significantly reduced root aphid populations, but not to levels which can be considered adequate control. Soil-dwelling beneficial arthropods remained largely unaffected by the seed-applied insecticide, except for a single ground beetle species (*Bembidion quadrimaculatum oppositum*) which showed higher activity in the untreated plots during one of the two years. Plant density had minimal impact on the aphids and beneficial arthropods.

Finally, this study also investigated the impact of tillage (conventional versus reduced tillage) on beneficial epigeal arthropods, and their associated ecosystem services (particularly weed seed consumption and predation of live prey). Overall, the results indicated a strong incentive for adopting reduced tillage practices for sugar beet production, based on increased activity of spiders, centipedes and rove beetles. Although prey consumption remained unaffected by the tillage system used, increased weed seed consumption was observed under the zone tillage system. This study also illustrated the importance of examining important beneficial taxa, such as ground beetles, on the species level rather than on the family level, based on the differing responses to tillage by different species.

## **DEDICATION**

I dedicate this work to my grandparents, Otto and Elize Conradie. Only through your lifelong hard work and unconditional devotion to your children and grandchildren have I been able to reach this milestone. For this I thank you dearly.

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## CHAPTER 1

### INTRODUCTION

#### **Sugar beet classification**

Beet (*Beta vulgaris* L.) is a temperate root crop belonging to the plant family Chenopodiaceae (Sauer 1993; Austin 2002; Milford 2006). Three subspecies of *B. vulgaris* are recognized, namely *B. vulgaris* ssp. *maritima* (L.), *B. vulgaris* ssp. *adanensis* (Pamuk.), and *B. vulgaris* ssp. *vulgaris* (Lange *et al.* 1999). The subspecies, *B. vulgaris* ssp. *vulgaris*, contains only cultivated varieties, of which sugar beet is one (Austin 2002; Francis 2006). Based on its use and external morphology, *B. vulgaris* ssp. *vulgaris* can be further divided into four groups, namely the garden beet group (*B. vulgaris* ssp. *vulgaris* var. *vulgaris*), the leaf beet group (*B. vulgaris* ssp. *vulgaris* convar. *cicla* Alef.), the fodder beet group (*B. vulgaris* ssp. *vulgaris* var. *rapacea* Koch), and the sugar beet group (*B. vulgaris* ssp. *vulgaris* var. *altissima* Döll) (Lange *et al.* 1999; Hammer 2001).

#### **Origin and domestication of cultivated sugar beet**

Beets were not originally grown for the purpose of extracting sucrose. In fact, considering the lengthy period of time that this plant has been associated with mankind, it is only relatively recently that its potential as a source of sucrose has been exploited extensively. Different varieties of beet were originally grown (at least 2000 years ago) as a garden vegetable to supplement human diets, albeit in a form very different from the modern-day sugar beet produced on a commercial scale (Draycott 2006; Francis 2006). These early garden versions of beet were most likely selected from wild *Beta* spp. (probably *B. vulgaris* ssp. *maritima*) endemic to the Mediterranean and Atlantic coasts of Europe (Sauer 1993; Vaughan and Geissler 2009).

The first extensive field production of beet, which occurred during the seventeenth century, was directed towards producing fodder for cattle (Francis 2006). Interestingly, several different types of *B. vulgaris* were cultivated for the purpose of feeding livestock, frequently having variously colored roots (Draycott 2006). By the mid-eighteenth century, a white-fleshed beet grown in Germany and Silesia (also as a livestock feed), was used to breed beets with a higher sugar content -- the ancestor of our modern-day sugar beet (Francis 2006). In 1802, a sugar beet factory in Cunern, Silesia was the first to produce sugar from this crop (Whitney and Duffus 1986). This led to the extensive spreading of the crop throughout Europe and to other parts of the world during the twentieth century (Draycott 2006), most notably to Argentina, Canada, Chile, and the United States (Whitney and Duffus 1986). Since it was discovered in Germany that sugar could be produced from this crop, the sugar beet industry has experienced many setbacks in both Europe and the United States (Francis 2006). Even though the first sugar beet factory in the United States was opened during 1838 in Northampton, Massachusetts, it wasn't until 1879 with the opening of a factory in Alvarado, California, that the industry took off in this country and became profitable (Whitney and Duffus 1986). In many countries, including the United States, the sugar beet industry is considered established with many advances having been made in improving the sugar beet crop. Such continuing improvements, for example, made it possible to produce beet with a sugar content of as high as 20% (Draycott 2006).

### **Sugar beet uses**

Sugar cane (*Saccharum officinarum* L.) and sugar beet are the two sources of sucrose derived exclusively from cultivated crops (Draycott 2006). Annually, approximately 165 million tons of sugar is produced from these two crops worldwide (Khan 2010). Although the amount of sugar produced from sugar cane exceeds that from sugar beet, sugar beet still accounts for roughly a quarter of sugar produced annually on a global scale (Draycott 2006; Khan 2010). In

fact, sugar beet is considered an important source of sucrose in temperate regions (Vaughan and Geissler 2009) where the production of sugar cane is not feasible.

The use of sugar beet is not restricted to sucrose production. Sugar beet roots, leaves (tops), and pulp (the remainder after sugar has been extracted) are used as livestock feed (Harland *et al.* 2006; Vaughan and Geissler 2009). Molasses derived from sugar beet can also be used as livestock feed (Harland *et al.* 2006) and in the production of industrial alcohol (Vaughan and Geissler 2009). In addition, syrup can also be produced from sugar beet. Recently, there has been increased interest in sugar beet as a biofuel crop (Cheesman 2004; Tzilivakis *et al.* 2005; Koga 2008; Dodić *et al.* 2009; İçöz *et al.* 2009; De Vries *et al.* 2010; Panella 2010; Foteinis *et al.* 2011; Maung and Gustafson 2011; Zeng *et al.* 2012).

### **Sugar beet production**

Climatic and edaphic factors dictate successful sugar beet production (Draycott 2006). Sugar beet is largely grown in the Northern Hemisphere (Oerke and Dehne 2004). Cultivation of this crop takes place in approximately 50 countries, located on five continents (Europe, North- and South America, Asia and North Africa) (Draycott 2006). On a global scale, approximately 227 million tons of sugar beet were harvested in 2008. The main producers of sugar beet are the European Union, the United States, the Russian Federation, Turkey, Ukraine, Iran, Japan, and China. In 2010, France, the United States, Germany, the Russian Federation, and Turkey, were listed as the top producers of this crop (FAOSTAT 2012, <http://faostat.fao.org>) (Figure 1.1).

The establishment of the sugar beet industry in the United States was challenging (Francis 2006). However, once the industry got a foothold in California, it expanded to include production in other areas of the country. Currently, eleven states in the United States produce sugar beet. Production in the Red River Valley of Minnesota and North Dakota constitutes 56% of the total sugar beet acreages, with beets also grown in the high plains areas east of the Rocky

Mountains, Pacific northwest states, California, and Michigan and surrounding area (Francis 2006; Fernandez-Cornejo *et al.* 2014). The United States uses approximately 12 million tons sugar per year (Khan 2010). Sugar cane typically accounts for 44% of the country's sugar requirements, while sugar beet constitutes the remaining 56% (Khan 2010).

### **Arthropod pests of sugar beet**

On a global scale, animal pests (both vertebrates and invertebrates) of sugar beet have been estimated to cause yield losses of approximately 6% (Oerke and Dehne 2004). As is the case with most other cash crops, a variety of insect pest species are associated with sugar beet (Whitney and Duffus 1986; Lange 1987; Hein *et al.* 2009). Some of these pests are of greater importance than others depending on geographical region, local ambient conditions, and production practices (Dewar and Cooke 2006; Hein *et al.* 2009). These pests represent several different insect orders and families. In general, damaging insects can be classified as root feeding or leaf feeding guilds, or a combination of the two (Dewar and Cooke 2006). Contained within these two functional groups are insects with differential feeding strategies i.e. chewing and sap-sucking insects. Aphids are soft-bodied, sap-sucking insects in the suborder Sternorrhynca (Hemiptera: Aphididae), and they use plant phloem for their nutritional needs. They have tremendous reproductive capabilities owing to their parthenogenetic mode of reproduction and telescoping of generations within the female (Johnson and Triplehorn 2004). A number of foliar feeding aphid species are reported feeding on sugar beet, namely *Myzus persicae* (Sulzer), *Aphis fabae* Scopoli, *Macrosiphum euphorbiae* (Thomas), and *Aulacorthum solani* (Kaltenbach) (Dewar and Cooke 2006; Dewar 2007). Some of these (e.g. *M. euphorbiae* and *M. persicae*) are considered economically important primarily due to their viral transmission capabilities, while others have a direct impact on sugar beet productivity and yield as a consequence of their sap-sucking habits, especially under high population densities.

Most of the approximately 4,700 aphid species that have been described (Remaudière and Remaudière 1997) are foliage-feeding, but some species have exploited plant roots as a viable ecological niche. Nonetheless, in terms of their damage potential, root-colonizing aphids are comparable to aphid species feeding on above-soil plant parts in their capacity to induce damage to the host (Van Dam 2009). One of the more interesting aphid genera utilizing sugar beet as a secondary host (a herbaceous plant used to complete development of parthenogenetic generations during summer), are root aphids in the genus *Pemphigus* Hartig. These aphids form colonies on sugar beet roots during the warmer months of the year and are classified as sap-sucking root feeders (Hein *et al.* 2009). Controlling root aphids presents a particular challenge due to their subterranean biology.

The sugar beet root maggot, *Tetanops myopaeformis* (Röder) and the sugar beet root aphid (SBRA) are considered the most serious root feeding pests of sugar beet in North America (Hein *et al.* 2009). A comprehensive overview on the biology and management of the SBRA, *Pemphigus betae* Doane, is presented in Chapter 2. However, no chemical products are currently registered for controlling the SBRA on sugar beet (Hein *et al.* 2009). In addition, the wide-scale use of pesticides has come under focus in response to public concerns regarding the hazards that these chemicals pose to human health and the environment, and the number of new pesticides developed is limited by stringent regulations and high costs (Dewar and Cooke 2006). For these reasons, there exists an urgent need to further investigate alternative SBRA management strategies in order to curtail the damage caused by this pest.

### **Sugar beet and weed competition**

Apart from invertebrate pests, sugar beet is also susceptible to weed competition that can contribute to substantial yield loss in this crop (May 2001; Gianessi 2005; May and Wilson 2006). In fact, weeds are considered the most important pests of sugar beet, accounting for yield

losses regardless of where the crop is grown (Oerke and Dehne 2004). Weeds have been a problem in sugar beet cultivation since production of the crop was first initiated by competing for moisture, inorganic nutrients, and sunlight (Oerke 2006). Weeds are a major limiting factor to sugar beet production worldwide, because the low-growing nature of the sugar beet plant makes it prone to being easily overgrown by taller weed species (Khan 2010). The slow growing nature of this crop early in the season also makes it susceptible to competition with weeds. Approximately 250 plant species have been declared as weeds globally, 24% of which have been recorded in sugar beet production regions (May and Wilson 2006). Of these, 70% are broad-leaf weeds and the remainder are grassy weeds. Broad-leaf annual weeds are considered the most competitive with sugar beets (May and Wilson 2006). Other than causing direct competition with the crop for resources, agricultural soils act as weed seed banks, with the seed bank being closer to the soil surface as cultivation intensity decreases (May and Wilson 2006).

With the decreased availability and high cost of hand labor, chemical control of weeds became imperative (May and Wilson 2006). Chemical weed control in sugar beet has been practiced since the 1950's (Schweizer and Dexter 1987), and it has traditionally relied on a sequence of pre- and/or post-emergence herbicide applications, often applied as tank mixtures to control a variety of weed species simultaneously (May 2001). The number of herbicide applications to the sugar beet crop within a single season could be substantial. Gianessi (2005) mentions an average of 11.7 herbicide treatments/acre, contributing significantly to input costs. Carlson *et al.* (2008) mention that a total of 3-4 herbicide applications is typical for the USA when using conventional herbicides that are often applied as tank mixtures. In many cases, mechanical weed control was also required in addition to the chemical control regime (May 2001; Khan 2010). Conventional herbicides could also be damaging to the crop itself (Märländer 2005).

Chemical control measures for broad-leaf weeds that do not necessitate the application of tank mixtures became feasible with the introduction of glyphosate-tolerant sugar beet varieties in

2008 (Khan 2010). These genetically modified varieties reduced the number of herbicide applications to an average of two applications per season in the USA (Khan 2010). Glyphosate [*N*-(phosphonomethyl) glycine], a post-emergence, foliar-applied herbicide, is considered the most important herbicide worldwide, and it is the most commonly applied herbicide in the USA (Duke and Powles 2008). The glyphosate molecule inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). This molecule is the only inhibitor of EPSPS (Duke and Powles 2008). For this reason, glyphosate was mostly used as a pre-plant herbicide, or in situations where direct contact with crops could be avoided (Duke and Powles 2008). However, gene (*CP4*) transfer techniques have led to the development of several glyphosate-tolerant crops, including canola, corn, cotton, soybeans, and sugar beet in the USA, allowing for post-plant treatment without the risk of crop damage. Some of these genetically modified crops were introduced as early as 1996 for commercial production in the USA. Since that time, there has been a phenomenal increase in acreages planted to these crops (Gianessi 2005; Gianessi 2008). Sugar beet was no exception to this, with North Dakota and Minnesota planting 50% of their crop to glyphosate-tolerant varieties, Michigan planting 55%, Colorado, Nebraska, and Wyoming planting 85-90%, and Idaho planting 98% in 2008 when these cultivars were made available for commercial production (Khan 2010).

The popularity and rapid wide-scale adoption of glyphosate-resistant crops can be attributed to several factors. Compared to many other conventional herbicides, it is less dangerous or harmful to the environment due to low vertebrate toxicity (Franz *et al.* 1997; Williams *et al.* 2000; Bennett *et al.* 2004; Cerderia and Duke 2006). In addition, the introduction of glyphosate-tolerant crops provided for improved weed control at reduced costs, less risk of crop damage resulting from the use of conventional herbicides, more efficient time management, and improved efficacy (Gianessi 2005; Gianessi 2008; Khan 2010). Taking sugar beet as an example, Gianessi

(2005) estimated that 100% adoption of glyphosate-tolerant sugar beet would result in an aggregated saving of \$93 million per annum in the USA.

Adopting glyphosate-tolerant crop technology also allows for reduced tillage (conservation tillage) where at least 30% crop residue is left covering the soil surface (Cavalaris and Gemtos 2002; Köller 2003; Givens *et al.* 2009; Fernandez-Cornejo *et al.* 2014). This is especially noteworthy for sugar beet production in western states of the USA where this practice was not possible prior to the introduction of glyphosate-tolerant sugar beet varieties due to weed pressure (Khan 2010). Conservation tillage practices include strip tillage, zone tillage, no-tillage, ridge tillage, and mulch tillage (Cavalaris and Gemtos 2002; Köller 2003). Practicing reduced tillage provides for certain benefits, such as improved soil management (e.g. less soil erosion caused by wind and water, and less soil degradation), increased moisture holding capacity of soils, and decreased machinery costs and carbon emissions (Cavalaris and Gemtos 2002; Håkansson *et al.* 2006; Dill *et al.* 2008; Duke and Powles 2008; Koger *et al.* 2009; Khan 2010; Fernandez-Cornejo *et al.* 2014). These benefits are exemplified by the fact that approximately 70% of the total sugar beet acreage in Nebraska was produced using a zone tillage system in 2009, only one year after the introduction of glyphosate-tolerant sugar beet varieties (Khan 2010). Reduced tillage does, however, also have its drawbacks. For example, an increased bulk density with depth (i.e. compaction) is considered a disadvantage of conservation tillage as this could reduce yield in sugar beet (Cavalaris and Gemtos 2002).

Unfortunately, repeated glyphosate applications following the introduction of genetically modified crops in the presence of few resistance management strategies have led to rapid resistance development in some weed species due to the increased selection pressure (e.g. Powles *et al.* 1998; Pratley *et al.* 1999; Lee and Ngim 2000; Culpepper *et al.* 2006; Powles and Preston 2006; Duke and Powles 2008; Legleiter and Bradley 2008; Powles 2008; Urbano *et al.* 2009; Khan 2010; Waltz 2010; Walker *et al.* 2011). Khan (2010) stated that these resistant weed species



(e.g. kochia) or the ones most likely to develop resistance are also the most problematic in sugar beet production systems. Some of these weed species (such as lambsquarters) can also act as a secondary host to the SBRA, potentially contributing to increased pest incidence (see Chapter 2 for further discussion on this issue). Currently, managing herbicide-resistant weeds, as well as preventing the development of resistance in glyphosate-susceptible weed populations, presents a significant challenge to producers (Shaner 2000; Weersink *et al.* 2005; Walsh and Powles 2007; Gustafson 2008; Neve 2008; Werth *et al.* 2008; Green 2009; Johnson *et al.* 2009; Sammons *et al.* 2009; Beckie 2011; Neve *et al.* 2011; Shaner *et al.* 2012). It is imperative to adopt and integrate weed management strategies that slow the selection pressures exerted by glyphosate if this technology is to remain viable in the future. Recommendations to slow resistance development in weed populations include good crop husbandry and rotation schemes, as well as improved management strategies (Duke and Powles 2008) that conserve natural enemies that feed on weeds.

### **Sustainable sugar beet pest and weed management**

Crop loss resulting from invertebrate pest and weed pressure can be substantial (Oerke and Dehne 2004; Oerke 2006). Large-scale monoculture cropping systems upsets the natural balance that functions to keep pests and weeds in check, thereby allowing some of these organisms to reach pest status. Oerke and Dehne (2004) estimated that yield loss in sugar beet due to weeds, animal pests (vertebrates and invertebrates), viruses, etc. would account for a staggering 80% of the global production in the absence of any crop protection strategies. In an attempt to circumvent these losses, control measures are usually taken in the form of physical, biological, or chemical measures (Oerke and Dehne 2004; Oerke 2006; Pedigo and Rice 2009). Under circumstances where chemical pest control becomes or is less effective, increasing attention should be given to alternative management strategies. This is usually done in the form of an integrated pest management (IPM) system.

The United States Department of Agriculture (USDA) has defined IPM as, “a management approach that encourages natural control of pest populations by anticipating pest problems and preventing pests from reaching economically damaging levels. All appropriate techniques are used such as enhancing natural enemies, planting pest-resistant crops, adapting cultural management, and using pesticides judiciously” (USDA 1993). However, in order to effectively integrate management strategies, a good understanding of pest and weed biology is imperative (Pedigo and Rice 2009). Pest management tactics, such as host plant resistance, cultivation practices, seed treatments, and the conservation of natural enemies, are components of an integrated approach to pest management, and constitute the main focus of this research. With sugar beet in particular, the use of alternative pest management tactics (e.g. biological control and the use of pest-resistant varieties) have been advocated to reduce pesticide applications (Dewar and Cooke 2006).

**Natural enemies.** Natural enemies of weed and invertebrate pests render an important service to human societies through ‘ecosystem services’ (De Groot *et al.* 2002). Four categories of ecosystem services have been categorized by Reid *et al.* (2005), namely cultural, provisioning, regulating and supporting. From a pest management point of view, regulating ecosystem services are viewed as the most essential. By integrating ecological services, such as pest- and weed seed removal by beneficial organisms, it is possible to attain a higher degree of pest- and weed management than might otherwise have been present in the absence of these organisms. Aphids, for example, are preyed upon by a variety of arthropod natural enemies, collectively known as aphidophaga (Völkl *et al.* 2007), preventing their populations from reaching damaging levels (Yepsen 1984). Some of the best known examples of these aphidophagous predators include spiders (order Araneae), centipedes (class Chilopoda), harvestmen (order Opiliones), as well as several different insect families. Prominent insect natural enemies of aphids include the Syrphidae, Chamaemyiidae, and Cecidomyiidae (Diptera); Anthocoridae and Nabidae

(Hemiptera); Chrysopidae and Hemerobiidae (Neuroptera); Staphylinidae, Coccinellidae, and Carabidae (Coleoptera), amongst others (Yepsen 1984; Flint *et al.* 1998; Schmidt *et al.* 2003; Snyder and Ives 2003; Brewer and Elliot 2004; Hajek 2004; Schmidt *et al.* 2004). In addition to these, several parasitic wasp families parasitize aphids (Yepsen 1984; Flint *et al.* 1998; Schmidt *et al.* 2003; Snyder and Ives 2003; Brewer and Elliot 2004; Hajek 2004; Schmidt *et al.* 2004). Detailed work concerning the natural enemy complex of many pest aphid species, as well as their impact on aphid populations, has been conducted for several crops, such as alfalfa, apples, bananas, cereals, and soybeans (e.g. Carroll and Hoyt 1984; Stechmann and Völkl 1990; Grasswitz and Burts 1995; Starý 1995; Ehler *et al.* 1997; Chen *et al.* 2000; Snyder and Ives 2003; Rutledge *et al.* 2004; Nielsen and Hajek 2005; Tomanović *et al.* 2008; Harwood *et al.* 2009; Dib *et al.* 2010; Gontijo *et al.* 2012). However, the overwhelming majority of such studies focus on above-soil aphid pest species, with little regard to subterranean aphids, such as *Pemphigus* spp. This is not surprising, considering the cryptic nature of root aphids and the difficulty involved in observing their colonies and/or the impact of predators on their populations. The probability that generalist, epigeal natural enemies will feed and possibly regulate root aphid populations, should not be overlooked.

Agroecosystems are subjected to high levels of disturbance through cultivation, often rendering them unfavorable towards natural enemies. However, arthropods such as rove beetles, ground beetles, centipedes, harvestmen, and spiders are considered generalist predators of aphids that are often plentiful and species-rich in agroecosystems (Weibull *et al.* 2003; Brewer and Elliot 2004; Eitzinger and Traugott 2011). These generalist natural enemies are effective predators of above-soil aphid species (Carroll and Hoyt 1984; Winder 1990; Dennis and Wratten 1991; Schmidt *et al.* 2003) and are considered important natural enemies of various pest species (Brewer and Elliot 2004; Eitzinger and Traugott 2011). A characteristic of generalist natural enemies is that they have the ability to respond to aphid populations before exponential growth of

these populations takes place (Hajek 2004). Furthermore, many generalist feeders are frequently encountered on- or below-soil level, where they are more likely to encounter and feed on root aphids and other edaphic soil-borne pests. A general lack of SBRA parasitoids and reports that soil-dwelling predators can reduce above-soil aphid populations (Schmidt *et al.* 2003; Schmidt *et al.* 2004), leads to the question of the possible contribution of these organisms towards SBRA management. A complete account of the work that has been done to investigate the natural enemy complex of the SBRA is presented in Chapter 2.

In addition to feeding on pests, many beneficial arthropods also feed on weeds, thereby contributing to sustainable weed management. Classical and augmentative biological control of weeds have been the most commonly used strategies for controlling both invasive and native weed species in several countries (Hajek 2004; May and Wilson 2006). However, none of these strategies have been employed against weeds occurring in sugar beet fields. In terms of the resident beneficial arthropod fauna, there are many species that can contribute to weed management through their granivorous feeding habits. As an example, ground beetles make an important contribution to ecosystem functioning by post-dispersal (i.e. weeds that have been shed from the parent plant) weed seed feeding (Barney and Pass 1986a; Cardina *et al.* 1996; Cromar *et al.* 1999; Honek *et al.* 2003; Honek *et al.* 2005; Mauchline *et al.* 2005; Honek *et al.* 2006; Lundgren *et al.* 2006; O'Rourke *et al.* 2006; Menalled *et al.* 2007; Shearin *et al.* 2007; White *et al.* 2007; Honek *et al.* 2009; Davis and Raghu 2010).

**Host plant resistance.** Host plant resistance to insect pests can be more effective than the use of pesticides, and may contribute to decreased pesticide input (Sharma and Ortiz 2002; Oerke and Dehne 2004). For this reason, host plant resistance is often viewed as an environmentally friendly and sustainable approach to pest management (Dogimont *et al.* 2010). In addition, producers do not need special skills for adopting this management strategy, and there are no financial costs for adopting host plant resistance (Panda and Khush 2010). Apart from the

obvious benefits associated with host plant resistance (e.g. decreased environmental and human health risks due to decreased pesticide usage, and decreased pesticide residue on the crop), it is often the first line of defense against invertebrate pests, such as in the case with the SBRA (Hein *et al.* 2009). Furthermore, there are often no direct adverse effects on natural enemies. The value of resistant varieties is exemplified by the fact that the SBRA's presence can go unnoticed until damage to the crop occurs. Breeding for host plant resistance against pests can be viewed as a crop improvement strategy by obtaining maximal yields through safeguarding the crop from pest damage (Panda and Khush 2010). Host plant resistance provides an excellent opportunity for integrating additional pest management strategies, such as the use of biological control agents. From an entomological standpoint, three mechanisms of host plant resistance towards pests are recognized, namely antixenosis, antibiosis, and tolerance. Antixenosis, also referred to as non-preference, deters pest infestation by altering the behaviour of the pest insect, while antibiosis reduces the development, growth and/or reproduction of the pest insect (Painter 1951). Tolerance refers to the ability of a host plant to support phytophagous insect feeding without loss of vigour (Dent 2000).

**Cultivation practices.** Oerke and Dehne (2004) mention that changes in cultivation practices can affect pest populations. Therefore, it is reasonable to expect that these changes might also affect potential natural enemies. Indeed, cultivation practices have been observed to impact natural enemy abundance (e.g. Robertson *et al.* 1994; Cárcamo 1995; Marasas *et al.* 2001; Motobayashi *et al.* 2006; Menalled *et al.* 2007), by either causing direct or indirect mortality (e.g. through decreased prey abundance). In general, soil tillage is performed in order to achieve more suitable growing conditions for the crop by reducing bulk density, improving aeration and water movement, improving water infiltration into the soil profile, and ensuring increased contact between soil particles and seed in the seedbed (Köller 2003). Furthermore, it also serves to control weeds. In fact, the development and spread of glyphosate-resistant weed populations in

sugar beet fields have once again necessitated the occasional use of tillage as part of a weed management regime (Duke and Powles 2008).

Primary tillage for sugar beet is commonly conducted by means of moldboard plowing; a practice commonly referred to as conventional tillage (Cavalaris and Gemtos 2002; Köller 2003; Håkansson *et al.* 2006). Although pest management might not be the primary objective of tillage, some control might be afforded through its use. Indeed, one of the control options mentioned for SBRA, is thorough tillage of fields just after harvest. This practice would also destroy weeds that act as secondary hosts for these aphids. It has been observed that reduced tillage systems can also give rise to increased arthropod pest incidence, as these organisms are protected in the soil from adverse environmental conditions and their natural enemies (Oerke and Dehne 2004). On the other hand, reduced tillage can contribute to increased biodiversity through the establishment of a more stable micro-habitat for many organisms such as beneficial organisms (e.g. predators and parasitoids) (Kladivko 2001). In light of these differing, the impact of different tillage systems on the predatory complex of the SBRA needs to be taken into account if tillage and natural enemies are to complement each other in lowering SBRA numbers. This is especially important when considering the shifts in cultivation practices that have taken place after the introduction of glyphosate-tolerant sugar beet varieties. Testing for differences in both aphid and natural enemy populations between tillage systems (e.g. conventional and conservation tillage) might illuminate which system is most likely to support natural enemies and, thereby, potentially regulate aphid populations.

**Planting density and seed treatments.** For quite some time, studies with different crops and pest assemblages have found that agricultural practices other than tillage, such as plant density (a cultural practice), might also impact both pest and natural enemy communities. For example, increased plant density has been shown to increase beneficial arthropod abundance in crops such as cabbage (Yamamura 1999). Increased plant density has also been shown to

decrease pest incidence (e.g. wheat stem sawfly, aphids, milkweed bugs, Mexican bean beetles, and rice water weevil) in crops such as field beans, oats, sugar beet, rice and wheat (Luginbill 1958; Davis 1966; Way and Heathcote 1966; Heathcote 1970; Ralph 1977; Turchin 1988; Thompson and Quisenberry 1995). Conversely, increased plant density has also been shown to increase the abundance of certain pest species (e.g. frit flies) in crops such as cabbage and oats (Adesiyun 1978; Yamamura 1999). Therefore, investigating the effects of cultural practices such as plant density in the case of sugar beet and the SBRA is important, because SBRA spreads between individual sugar beet plants by means of cracks in the soil that form as the root increases in size (see Chapter 2). It can be hypothesized that closely spaced plants will lead to more extensive soil cracking, readily linking individual plants and, thereby, increasing access to nearby hosts for the SBRA. Conversely, extensive soil cracking between plants could also lead to improved access to SBRA colonies to potential natural enemies of the SBRA. Furthermore, changing plant density could also alter the degree of shading on the soil surface, potentially changing the soil microclimatic conditions (e.g. soil temperature and moisture). It is reasonable to suggest that these changing conditions could influence pest and natural enemy assemblage due to the microclimatic preference exhibited by different species. As an example, many ground beetle species are xerophilic or euryhygric, preferring agroecosystems that have a higher soil temperature (Thiele 1977). In addition, sugar beet is challenging to establish and it is important to know how differing plant density affects these organisms, even when plant density is not manipulated as a pest management strategy.

Another possible tool for SBRA management is the use of insecticide seed treatments. In sugar beet, the focus of insecticide seed treatments has been against foliar aphid pests and the diseases they transmit, although some work has been conducted on the impact of seed-applied insecticides on the SBRA (Strausbaugh *et al.* 2010). However, more research is needed on the impact of neonicotinoid seed treatments, not only on the SBRA, but also on beneficial arthropods.

For example, previous studies have shown that seed-applied insecticides, such as neonicotinoids, can have an adverse impact on natural enemies of crop pests (Mullin *et al.* 2005; Moser and Obrycki 2009; Seagraves and Lundgren 2011).

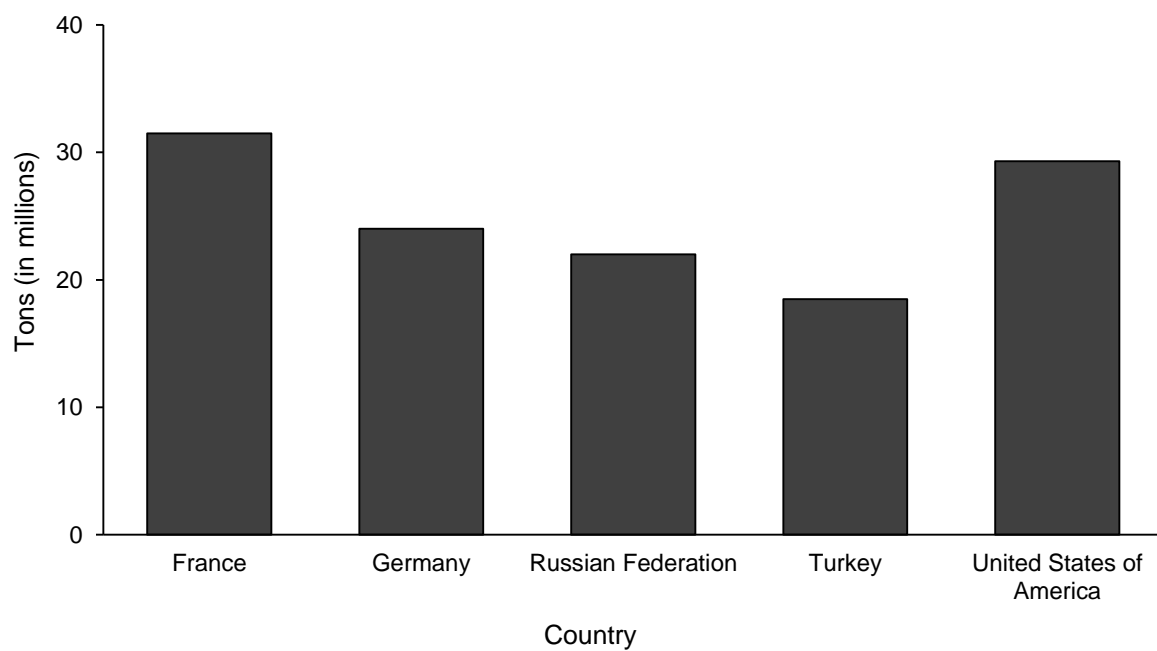
### **Research objectives**

For SBRA it would be worthwhile investigating how natural enemies, and host plant resistance can be integrated as a possible means of lowering sugar beet root aphid numbers. Chemical control of this pest is not practical, necessitating the use of alternative means of management that may be more ecologically sound. From a sugar beet production point of view, one possibility might be a more careful investigation of the interactions between sugar beet root aphids, new sugar beet varieties resistant to herbicides (e.g. Roundup (glyphosate)), the physical cropping environment (e.g. tillage practices), and the effect of resident epigeal natural enemies of these aphids and weed populations. Interest in these types of interactions involving other crops (e.g. wheat) has received increasing attention, particularly in North America (Brewer and Elliot 2004). With this in mind the specific objectives of this research were as follows:

- To review the life cycle, economic significance, and management of the SBRA (Chapter 2),
- To evaluate resistance of several glyphosate-tolerant sugar beet varieties to SBRA under field conditions and quantify the epigeal beneficial arthropod community across these treatments (Chapter 3),
- To quantify the influence of sugar beet plant density and seed treatments (specifically Poncho Beta) on SBRA infestations, as well as their effects on beneficial arthropods (Chapter 4),
- To evaluate the impact of tillage (conventional tillage vs. zone tillage) on beneficial soil-dwelling arthropods and the impact of these two tillage systems on the degree of



ecosystem services (specifically pest and weed seed removal) rendered by these organisms (Chapter 5).



**Figure 1.1:** Sugar beet production by the top five global producers in 2010 (From: FAOSTAT 2012, <http://faostat.fao.org>).

## CHAPTER 2

### THE SUGAR BEET ROOT APHID, *PEMPHIGUS BETAE* DOANE (HEMIPTERA: APHIDIDAE): BIOLOGY, ECONOMIC SIGNIFICANCE, AND MANAGEMENT STRATEGIES

#### Introduction

Aphids of the genus *Pemphigus* Hartig (Hemiptera: Aphididae: Eriosomatinae) form galls on the leaves of their primary host, *Populus* spp. L. (Salicaceae), but the summer morphs are mostly restricted to the roots of secondary hosts (Cranshaw 2004; Blackman and Eastop 2006; Footitt *et al.* 2010). This genus contains more than 70 named species, and 21 are known from North America (Blackman and Eastop 2006). Despite several species having been formally described, there still exists a degree of uncertainty over the true identity of the various *Pemphigus* spp. (Footitt *et al.* 2010). The difficulty in differentiating between different *Pemphigus* spp. based on morphological characters alone was already elucidated by Harper (1963). Blackman and Eastop (2006) mentioned that the identification of apterous aphids found on the roots of their secondary hosts is very difficult, if not impossible, when only morphological characters are used. Recent molecular analysis by Footitt *et al.* (2010) found that different *Pemphigus* spp. might co-exist on the same primary host with no visible distinction between the galls they form. However, despite the apparent difficulty in separating these species on a morphological base, certain crops (serving as secondary hosts) are known to harbor specific species, and have been the focus of extensive research due to the economic damage they inflict. One such species, the sugar beet root aphid, *Pemphigus betae* Doane (Hemiptera: Aphididae), is a sporadic pest of sugar beet (*Beta vulgaris* var. *vulgaris*) in all major sugar beet production regions of North America (Harper 1963; Hutchison and Campbell 1994; Campbell and Hutchison 1995a and b). This species was

originally described by Doane (1900) from Washington in the USA. This chapter investigates the biology, economic significance, and management strategies of *P. betae* as a pest of sugar beet.

### **Description of the sugar beet root aphid**

Sugar beet root aphids are small oval-shaped, pale yellowish insects, with a body length ranging from 1.9-2.4 mm (Blackman and Eastop 2006). Individual aphids secrete a waxy material, giving their subterranean colonies a moldy appearance (Blackman and Eastop 2006; Dewar and Cooke 2006; Hein *et al.* 2009; Natwick 2010) (Figure 2.1 a and b). It is thought that this waxy substance plays a role in repelling moisture (Hein *et al.* 2009). Additionally, it may be possible that the wax also protects the aphids from their own honeydew, because ant attendance appears to be low/absent, while their cauda (used for flicking honeydew away from the aphid) are reduced. Harper (1963) noted that these aphids cover their honeydew with wax while still enclosed in a gall on the primary host. The amount of wax present on a sugar beet root is useful for rating sugar beet root aphid infestation levels in the field (Hutchison and Campbell 1994). In contrast to most other aphid species found above-soil level, soil dwelling morphs of *P. betae* have reduced appendages (siphunculi, legs, antennae, and cauda) as an adaptation to their subterranean existence (Dewar and Cooke 2006).

### **Primary and secondary host plants**

As with most host-alternating aphid species, the sugar beet root aphid is restricted in its primary host range, but the secondary host range is more diverse. *Pemphigus* spp. prefer balsam poplars in the genus *Populus* (Figure 2.2) as their primary hosts (Blackman and Eastop 2006). Over the years, several *Populus* spp. have been implicated to harbor overwintering *P. betae* eggs in North America, the most common being the narrowleaf cottonwood, *P. angustifolia* James (Parker 1915; Harper 1963; Whitham 1978; Dewar and Cooke 2006; Hein *et al.* 2009), and the balsam poplar, *P. balsamifera* L. spp. *balsamifera* (Parker 1914; Parker 1915; Harper 1963; Hein

*et al.* 2009). The black cottonwood, *P. balsamifera* L. spp. *trichocarpa* (Hein *et al.* 2009) also serves as a primary host for this aphid. Although there is some overlap in their geographical distribution, these primary hosts occupy distinct regions in northern North America. For example, narrowleaf cottonwood is found primarily in the Rocky Mountain regions, the balsam poplar in the northern Rockies into Canada, extending its range to the Great Lakes area, and the black cottonwood having a range stretching from Alaska, through western Canada and into the northwestern USA, down as far south as Baja California (USDA 2014).

It is the roots of the secondary hosts which serve as the site of colonization by these aphids. Secondary hosts for *P. betae* include sugar beet, common lambsquarters (*Chenopodium album* L.), kochia (*Kochia scoparia* (L.)), *Rumex* L. spp. and pigweed (*Amaranthus* L. spp.) (Blackman and Eastop 2006; Hein *et al.* 2009). In addition to these, Harper (1963) reported that laboratory-reared *P. betae* established colonies on Swiss chard and red beets, spinach (*Spinacia oleracea* L.), and alfalfa (*Medicago sativa* L.). However, alfalfa proved to be a poor host for this aphid species.

### **Sugar beet root aphid life cycle**

For the pest manager and producer alike, a basic understanding of the life cycle of the sugar beet root aphid is essential in considering management options (Figure 2.3). *Pemphigus betae* has a complex and varied life cycle, as observed by Parker (1914) and Harper (1963). This aphid is usually heteroecious and holocyclic (Blackman and Eastop 2006), but anholocyclic apterae are known to overwinter in the soil and initiate new colonies with the development of favorable weather conditions (e.g. as is the case in Minnesota where most new infestations are reportedly initiated by these morphs) (Moran and Whitham 1988; Hutchison and Campbell 1994).

Harper (1963) observed that an overwintering egg (Figure 2.4 a) hatches into an apterous, viviparous, parthenogenetic fundatrix (Figure 2.4 b) during spring (late April to early May in

southern Alberta). Her initial feeding on the dorsal surface of a leaf from the primary host causes the leaf tissue to form a protective gall along the mid-rib (Figure 2.5 a and b). Enclosed within her gall, the fundatrix subsequently produce female alate, viviparous, parthenogenetic fundatrigeniae (Figure 2.4 c) that, when mature, migrate from the primary to the secondary host during early to midsummer (late June to mid-August in southern Alberta). Harper (1963) reported the average reproductive capacity of a fundatrix at 163 offspring, and Parker (1914) estimated it to be 75. By dissecting 10 fundatrigeniae, Harper (1963) found that the average reproductive potential for each female was 13.3 aphids. After locating a suitable secondary host, the fundatrigeniae subsequently give birth to apterous, viviparous, parthenogenetic alienicolae (Figure 2.4 d). This stage in the life cycle of the sugar beet root aphid reproduces on sugar beet roots; therefore they are responsible for economic damage observed in the crop. Harper (1963) established the optimum soil temperature for reproduction by alienicolae to be 25-27°C.

Several generations of alienicolae are produced (up to seven) before they eventually produce alate, viviparous, parthenogenetic sexuparae (Figure 2.4 e) that migrate back to the primary host beginning in late August and into fall. Moran *et al.* (1993) were able to determine that a combination of low temperature and crowding leads to the production of sexupara. These sexuparae, in contrast to most other aphid species, can only produce apterous males and oviparae (which are larger than the males), known as sexuales. The average reproductive capacity of the sexupara is estimated at six individuals per female (Harper 1963), all being apterous with reduced mouth parts. After mating with the apterous males, sexuales produce an overwintering egg (one per female) on the primary host. This is the only sexual generation in the life cycle of *P. betae*. Eggs are deposited in crevices of the bark where they are protected from ambient conditions throughout the winter. Harper (1963) reported that the egg is deposited in a white waxy secretion and initially appears white, but it turns darker as it ages. Often, however, the female dies with the

egg still inside her body (Parker 1914; Harper 1963). The eggs have an obligatory diapause and Harper (1963) recorded the eggs hatching in late April to early May in southern Alberta.

### **Economic significance of the sugar beet root aphid**

Economically damaging infestations of this pest have been reported from several locations in North America. Aliencolae are reported to prefer the hosts' secondary roots as their site of feeding (Harper 1963; Summers and Newton 1989; Hein *et al.* 2009; Natwick 2010), but heavy infestations may spread to the tap root giving it a rubbery and flaccid appearance under conditions of extreme stress and inadequate moisture levels (Harper 1963; Winter 1999; Hein *et al.* 2009). Root aphid damage is often most severe under drier conditions (Summers and Newton 1989; Hutchison and Campbell 1994; Winter 1999). Harper (1963) also noticed that damage from early frost might be more severe for root aphid-infested beets.

Large populations of *P. betae* can induce significant reductions in yield (Harper 1963; Summers and Newton 1989; Hein *et al.* 2009), sugar content (Harper 1963; Hutchison and Campbell 1994; Winter 1999; Hein *et al.* 2009), and recoverable sugar (Summers and Newton 1989; Hutchison and Campbell 1994). *Pemphigus betae* infestation levels exceeding a value of 2.0 on the at-harvest root rating index (see scouting procedures below) are reportedly required to cause consistent loss in recoverable sugar, although precipitation levels can also influence this (Hutchison and Campbell 1994). Under conditions of extreme stress and heavy infestations, the alienicolae can induce stunting, chlorosis, wilting, and even death of sugar beet plants in response to their feeding (Harper 1963; Summers and Newton 1989; Dewar and Cooke 2006; Natwick 2010). This can be ascribed to the interference with moisture and nutrient uptake (Summers and Newton 1989; Hein *et al.* 2009). No economic threshold levels have been established for sugar beet root aphid on sugar beet, and Hutchison and Campbell (1994) mention that the sporadic nature of this pest, coupled with its cryptic biology, has hindered extensive research on its

economic significance as a pest of sugar beet. However, it is known that even moderate infestation levels can induce sugar loss of up to 30%, when above-soil symptoms are absent (Summers and Newton 1989; Hein *et al.* 2009). Yield loss of 36-60%, depending on the level of root aphid infestation, have been reported by Summers and Newton (1989) in California, while Hutchison and Campbell (1994) reported yield losses of 32%. Furthermore, Hutchison and Campbell (1994) also recorded losses of 31% and 54% in sugar content and recoverable sugar, respectively, as a result of root aphid infestations. Hutchinson and Campbell (1991) reported that in 1984 and 1989 sugar beet root aphids infested 10% of 33,000 ha sugar beets in southern Minnesota, leading to losses of \$3 million.

A study conducted in Minnesota by Hutchison and Campbell (1994) found that field infestations of *P. betae* tend to occur in elliptical foci within fields. A similar phenomenon was observed by Summers and Newton (1989) in California. However, this does not seem to be the norm for many locations (Hein pers. communication), as these foci likely develop as a consequence of sparse initial infestations which subsequently establish well in the field. Hutchison and Campbell (1994) mentioned that severe infestations could result in individual foci merging with each other, leading to larger infestations. The formation of these foci could be ascribed to the tendency of these aphids to move down rows, rather than across them (Summers and Newton 1989). Sugar beet root aphids are known to use cracks in the soil to migrate between individual hosts (Harper 1963), but they can also be dispersed via precipitation, irrigation water, wind and field equipment (Harper 1963; Summers and Newton 1989; Hutchison and Campbell 1994). Therefore, variability in soil type might play a role in the formation of these foci.

### **Sugar beet root aphid surveillance**

Accurately establishing population densities for sugar beet root aphids presents a challenge, because the economic important stage of this insect is subterranean. Because root



aphid feeding does not always produce above-soil symptoms (e.g. Winter 1999), and because individual aphids are so small, it is important to dig up roots and examine them for aphids and the presence of the characteristic waxy material. For this reason, Hutchison and Campbell (1994) developed an at-harvest root rating index in Minnesota in 1990. The index ranges in scale from 0-5, with 0 signifying no infestation, while a 5 indicates the highest level of infestation achievable. This scale relies largely on quantifying the amount of aphid-secreted wax present on the root, rather than making direct aphid counts. Apart from its usefulness in rating root aphid infestation levels, this rating scale is also helpful when evaluating cultivars for sugar beet root aphid resistance (Hutchison and Campbell 1994). However, despite the development of the at-harvest root rating index, there still exists the need to effectively monitor sugar beet root aphid migration into sugar beet fields and their subsequent population growth (Hutchison and Campbell 1994). In an attempt to investigate population growth and age-specific life tables of *P. betae*, Campbell and Hutchison (1995a) used hydroponic growth pouches to rear these aphids. Their method allowed the authors to determine the fecundity of individual root aphids, something that would be challenging under field conditions. This method proved successful judging by the fact that the authors could maintain a culture for over three years.

### **Management practices**

Root aphids are especially difficult to control by means of conventional insecticides (Winter and Patrick 1997; Dewar 2007) due to their cryptic biology. Most systemic insecticides cannot be transported downward towards the root system of the host plant (Dewar 2007), while early-season chemical control (such as seed treatments) are reported to be inconsistent against aphids moving into fields later in the season (Dewar 2007; Hein *et al.* 2009). However, some chemicals, such as triazamate (no longer registered in the United States), were used to control root aphids (*P. bursarius* (L.)) on lettuce (Parker *et al.* 2002) and is also effective against the sugar beet root aphid (Hein pers. communication). Results from chemicals tested in the US, such

as terbufos (Counter 15G), were inconsistent (Campbell and Hutchison 1991), while others, such as chlorpyrifos (Lorsban 4E, 15G) led to increased root aphid populations (Campbell and Hutchison 1991). Harper (1961) also found that the use of insecticides reduced predator numbers significantly, leading to a loss of biological control which was equally effective in reducing root aphid numbers compared to the insecticides. Another option for chemical control of this pest might be the use of soil drenches (Winter 1999). In 1989, Summers and Newton reported that there are no registered chemicals for use against this aphid, and this situation has not changed (Hein *et al.* 2009). In addition to soil drenches, seed treatments with neonicotinoids have also shown some promise (Strausbaugh *et al.* 2010).

Due the importance of sugar beet as a source of sucrose and as a rotational crop, and the absence of reliable chemical control measures, there is a need for alternative control measures of SBRA to curb the economic losses. Possible approaches for sugar beet root aphid management include biological control, cultural control, and host plant resistance. Interest in the integrated use of these management strategies in other crops (e.g. wheat) has received increasing attention in North America (Brewer and Elliot 2004), and can be implemented to the benefit of sugar beet production.

**Host plant resistance.** Breeding sugar beet for resistance to various pests and diseases has been crucial in ensuring sustained cultivation in regions where these problems are pronounced (Bosemark 2006). The significance of using resistant or tolerant varieties for keeping pests and diseases at bay is evident when considering the possibility of increased yields with decreased chemical inputs (and hence, decreased production costs). The success rate for breeding pest resistant varieties of sugar beet, in general, has been low compared to breeding disease resistant varieties (Bosemark 2006). However, several sugar beet varieties show good resistance against the sugar beet root aphid.

In the past, several sugar beet varieties have been successfully screened for resistance against the sugar beet root aphid in North America (Harper 1964; Wallis and Turner 1968; Campbell and Hutchison 1995b; Winter 1999). In fact, resistance in some varieties was so pronounced that no aphids were able to survive (Campbell and Hutchison 1995b). The mechanisms underlying this resistance were ascribed to both antibiosis and antixenosis (Campbell and Hutchison 1995b). Currently, host plant resistance is considered the most important management strategy against the sugar beet root aphid (Hein *et al.* 2009). It is also highly probable that host resistance will continue to play an important role in sugar beet root aphid management with the availability of gene transfer techniques and marker-assisted selection which could contribute towards breeding disease and pest resistant varieties with higher yield (Bosemark 2006).

**Natural enemies of the sugar beet root aphid.** The inability to effectively control sugar beet root aphids with conventional chemicals might indicate that the value of natural enemies in suppressing their numbers could be high. In fact, prior to the use aphid resistant varieties, Harper (1963) indicated that natural enemies are largely responsible for curtailing sugar beet root aphid populations. Due to the fact that this is an indigenous pest to North America, it can be expected that native natural enemies may be more effective in suppressing their numbers. Therefore, conserving these beneficials could be important. However, with the damaging stages being subterranean, it is unlikely that parasites will play a significant role in reducing root aphid populations (Dunn 1960).

Although there is ample information on the predatory arthropod complex that prey on aphids occurring above ground, the opposite is true for subterranean aphid populations. It is likely that these aphids have a different predatory complex than that of their above-soil counterparts (Dunn 1960). Harper (1963) reported the flies, *Syrphus bigelowi* Curran, *Leucopis pemphigae* Malloch, and *Thaumatomyia glabra* (Meigen); the anthocorid bug, *Anthocoris antevolens* White;

and a coccinellid in the genus *Scymnus*, as predators of *P. betae* in Alberta. However, with the exception of *T. glabra*, these predators were all reported from the galls on the primary host, and not on subterranean aphid populations (Harper 1963). Dunn (1960) reported syrphid fly larvae and anthocorid bugs (*A. nemorum* (L.) and *A. nemoralis* (Fabricius)) preying on the closely related *P. bursarius* (L.) in their galls on the primary host. The coccinellids, *Coccinella transversoguttata* Faldermann, *C. trifasciata* Mulsant, and *Adalia bipunctata* Say, as well as the anthocorid bug, *A. antevolens*, prey on sexual forms and fall migrants of *P. betae* occurring on the primary host (Harper 1963). Similarly, Dunn (1960) noted three coccinellid species preying on sexuparae of a related species, *Pemphigus bursarius* (L.), arriving on poplars from their secondary host, lettuce.

In contrast to these, *T. glabra* prey on *P. betae* colonies in the soil, as reported in Alberta, California, and elsewhere (Dunn 1960; Harper 1963; Summers and Newton 1989). Other predators observed preying on subterranean sugar beet aphids include *Metasyrphus pauxillus* (Williston), *Hippodamia convergens* Guerin, *Scymnus collaris* Meish, and *Scymnus appaculus* Horn (Maxson 1916). Both Dunn (1960) and Rivard (1964) mention that there are many carabids and staphylinids associated with *P. bursarius* colonies below soil, and that these likely prey on the summer morphs. However, no studies have been conducted to determine the importance of these generalist predators on sugar beet root aphid populations, despite much emphasis given on the value of carabid beetles as generalist predators in other cropping systems (Eitzinger and Traugott 2011).

**Cultural practices.** Alternative management strategies that have been suggested to lower root aphid populations include sanitation, crop rotation, managing alternative hosts, and maintaining adequate soil moisture regimes (e.g. Summers and Newton 1989). Performing crop rotation can decrease the survival of alienicolae overwintering in the field, provided adequate weed control can be achieved. Weed species such as common lambsquarters and redroot

pigweed, acting as secondary hosts, can be abundant in sugar beet agroecosystems (and field margins) and need to be managed. If crop rotation is practiced, these alternative hosts should also be managed vigorously in the non-beet crop. Unfortunately, herbicide-resistant weed populations can pose a problem in this regard, especially with the availability of glyphosate-tolerant sugar beet varieties in which a single active ingredient is used to curtail weed populations.

It is also suggested that these aphids can be spread between fields on infected equipment (Summers and Newton 1989), highlighting the importance of practicing good sanitation. The use of frequent and early irrigation, coupled to maintaining high levels of soil fertility, has been suggested to keep sugar beet root aphid damage to sub-economic levels (Parker 1915; Harper 1964). Summer and Newton (1989) also suggest that tail water from fields infested with these aphids should not be applied to uninfested fields, because these aphids are readily transported by irrigation water. Dry soil conditions should be minimized where irrigation is applied, because root aphid populations build up rapidly under these conditions (Hutchison and Campbell 1991), and because it reduces water stress in the sugar beet crop. In addition to these, cultivation might also reduce sugar beet root aphid numbers. Thorough cultivation following harvest will destroy overwintering aliencolae in the soil in fields (Summers and Newton 1989).

### **Conclusions**

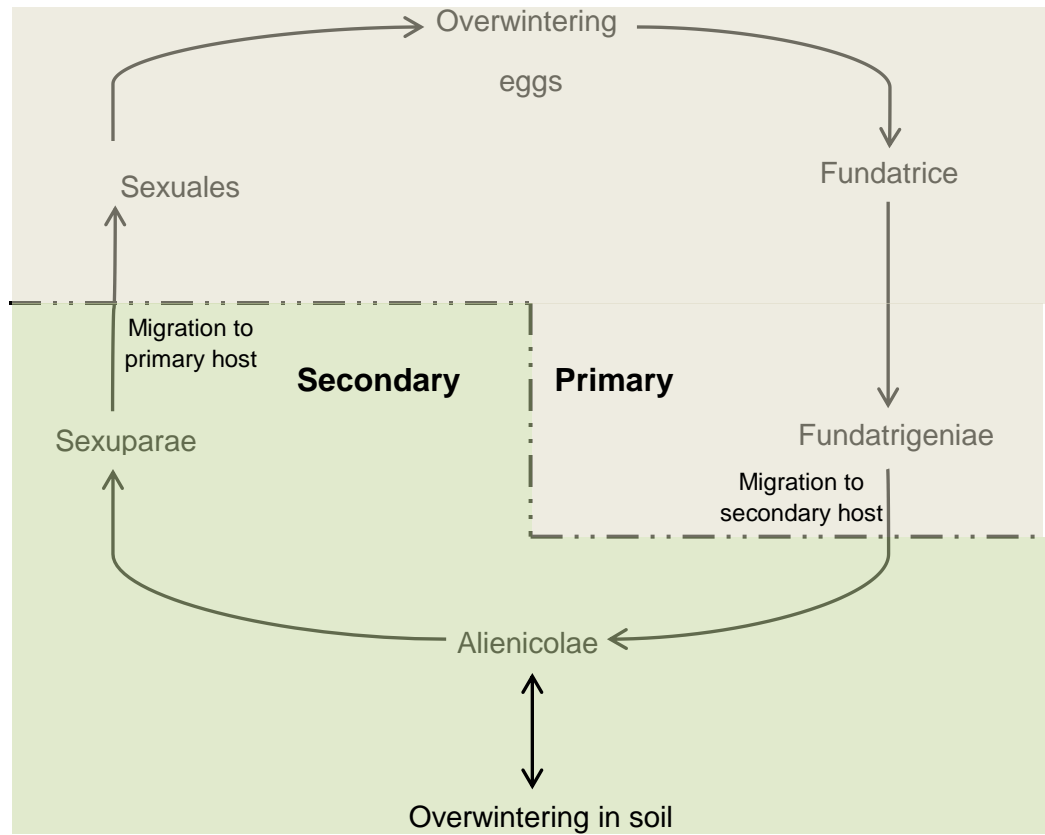
Even though it is considered a sporadic pest, the sugar beet root aphid poses a serious threat to sugar beet production in the main sugar beet production regions of North America due to its ability to reduce sugar content and yield. Control and detection of this aphid pest is not easily attained owing to the subterranean summer morphs occurring on the roots of the crop. Unlike most aphid pest species, chemical control of this aphid genus is not a viable option at present, and attention to alternative management strategies should take precedence. In this regard, host plant resistance, cultural techniques, and likely biological control, play a pivotal role.



**Figure 2.1:** Waxy filaments secreted by individual aphids (a) gives the colony a moldy appearance (b). (Photos: R.J. Pretorius).



**Figure 2.2:** *Pemphigus* spp. prefer poplars in the genus *Populus*, such as this narrowleaf cottonwood (*P. balsamifera* L.) as their primary host. (Photos: R.J. Pretorius).

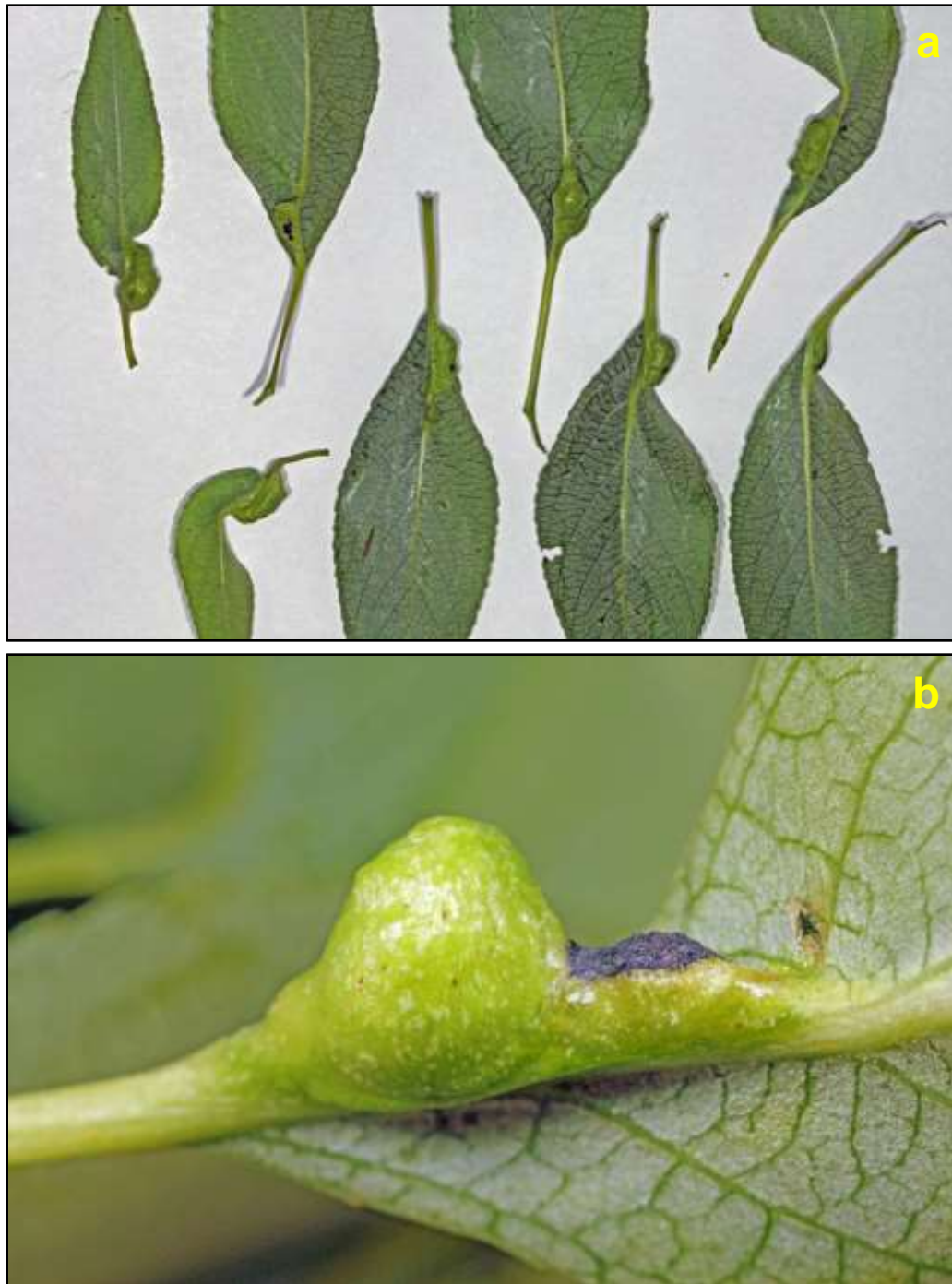


**Figure 2.3:** Graphical representation of the life cycle of the sugar beet root aphid, *Pemphigus betae* Doane. (Redrawn from Harper 1963).





**Figure 2.4:** Different life stages and morphs found in the life cycle of *Pemphigus betae*. Pictured are eggs (a), a fundatrix in a gall (b), fundatrigeniae in various stages of development in a gall (c), alienicolae on sugar beet roots (d), and sexupara amongst alienicolae on a sugar beet root (e). The eggs could not be confirmed with certainty as belonging to *P. betae*, but dead bodies of both sexuparae and sexuales were observed on the bark of the trees from which the eggs were collected. Furthermore, an aphid hatched from one of these eggs, but died in the absence of its host. The white filamentous material surrounding the eggs also conforms to the description given by Harper (1963). (Photos: R.J. Pretorius).



**Figure 2.5:** Narrowleaf cottonwood leaves with galls formed by the fundatrix of the root aphid, *Pemphigus betae* (a). A close-up of one of these galls which are usually located near the midrib of the leaf (b). (Photos: R.J. Pretorius).

## CHAPTER 3

### VARIETAL RESISTANCE OF GLYPHOSATE-TOLERANT SUGAR BEET TO THE SUGAR BEET ROOT APHID (HEMIPTERA: APHIDIDAE) AND THE RESPONSE OF EPIGEAL NATURAL ENEMIES

#### Introduction

The sugar beet root aphid (SBRA), *Pemphigus betae* Doane, is a sporadic pest of sugar beet wherever the crop is grown in the USA (Harper 1963; Hutchison and Campbell 1994; Campbell and Hutchison 1995 b). This species has a heteroecious, holocyclic lifecycle (Blackman and Eastop 2006), but anholocyclic apterae (overwintering in the soil) have been reported (Moran and Whitham 1988; Hutchison and Campbell 1994). In contrast to most aphid species, it is a subterranean pest, feeding on the roots of its secondary hosts. As a consequence of their feeding, SBRA infestations cause stunting, chlorosis, wilting, and sometimes even death of the host (Harper 1963; Summers and Newton 1989; Dewar and Cooke 2006; Natwick 2010). Due to its subterranean biology, management of this aphid is complicated, with no registered chemicals currently available for its control. Therefore, aphid-resistant sugarbeet varieties offer the most important means of curtailing sugar beet root aphid populations (Hein *et al.* 2009).

Sugar beet is vulnerable to weed competition (Scott *et al.* 1979; Francis 2006; May and Wilson 2006) as evidenced by reduced sugar content, root weight, and yield (Dewar *et al.* 2000). Yield loss can occur at low weed populations when weeds compete with the crop for moisture, sunlight, and nutrients (May and Wilson 2006). If left unchecked, weeds can reduce yields by 26-100% (Schweizer and Dexter 1987; May 2001). Beets are especially susceptible to competition from weeds up to the eight-leaf stage (May 2001). Before the introduction of monogerm seed, weed control in sugar beets involved relatively expensive and labor intensive techniques, such as

mechanical tillage, hand pulling, and hoeing, and more recently, mixtures of different herbicides (May 2001; May and Wilson 2006). Herbicide tank mixtures were necessary because no single herbicide could effectively control the diversity of weed species present in sugar beet fields without damaging the crop (Miller and Fornstrom 1989). In addition, many herbicides were ineffective at controlling weeds beyond the first true leaf stage (May 2001).

Glyphosate-tolerant sugar beet varieties were introduced in 2008, and by 2009 there was an estimated 85% adoption rate of this new technology (Khan 2010). A high adoption rate of these new varieties is largely due to the use of glyphosate, applied two or more times during the season (May 2001; Coyette *et al.* 2002; Dewar *et al.* 2002; Dewar *et al.* 2003). Additionally, there is less early-season crop damage compared to some of the traditional herbicide tank mixtures (Wilson *et al.* 2002). Reduced phytotoxicity early in the season resulted in improved crop vigour with early canopy closure, increasing competition with weeds, and subsequently increased sucrose yield (Wilson and Smith 1999). Compared to conventional herbicide applications, weed control with glyphosate can yield similar or even better results (Dewar *et al.* 2003). These genetically modified varieties also allow for reduced tillage practices, because weeds can be suppressed without the need for additional cultivation operations (May 2001; May and Wilson 2006). For producers, this translates into both input- and machinery-costs savings, as well as time savings (May 2001; Kniss *et al.* 2003; May 2003).

With the availability of new glyphosate-tolerant sugar beet varieties, it is imperative to evaluate the level of SBRA resistance amongst these varieties (Winter 1999). This is especially crucial in light of the fact that there is a high risk that some weedy plant species will develop resistance to glyphosate. Some of these plants are also hosts to SBRA and may be a source of infestation for the sugar beet crop. As an example, glyphosate resistant weed populations have already been observed in the sugar beet production regions of western Nebraska (Shaw *et al.* 2011). Ideally, initial screening for resistance against SBRA should be completed under

controlled conditions, such as in greenhouses, but subsequent field evaluation is recommended, because results may vary with environmental conditions (Campbell and Hutchison 1995 b).

In addition to host-plant resistance, other control options need to be considered as part of an integrated approach to SBRA management. Very little is known about the biological control of SBRA; however, there is evidence that these aphids are preyed upon in their galls on the primary host (Dunn 1960; Harper 1963). The chloropid fly species, *Thaumatomyia glabra* (Meigen), has been reported to reduce SBRA populations on sugar beet (secondary host) during the growing season (Dunn 1960; Harper 1963; Summers and Newton 1989; Hein *et al.* 2009). Harper (1964) stated that biological control of SBRA in the field is important for regulating aphid populations at densities below economic damaging levels.

Generalist natural enemies have the potential to control pests in temporary agroecosystems (Ehler and Miller 1978; Weidenmann and Smith 1997) and, unlike specialist predators, they can suppress pest outbreaks because they are present within the cropping system when pests arrive. Furthermore, they are present in fields throughout the cropping season, albeit likely in different species assemblages. To be considered effective, natural enemies in temporal agroecosystems should be able to rapidly colonize disturbed habitats, exhibit temporal persistence to maintain high population levels in the absence of the pest, and be opportunistic in order to exploit the target pest (Ehler 1990). These attributes are considered benefits for generalist natural enemies. In light of the limited management strategies, tactics, and tools for SBRA, conservation biological control deserves greater attention. However, little is known about the community assemblage and impact of generalist natural enemies of the SBRA in sugar beet agroecosystems in North America.

The objective of this study was to evaluate the level of SBRA resistance for several glyphosate-tolerant sugar beet varieties under field conditions in western Nebraska. The impact of

varietal resistance on aphid presence and the impact of these aphids on crop yield parameters were determined. A second objective of this study was to determine the community assemblage and response of predatory epigeal arthropod populations to SBRA infestations in varieties with different levels of SBRA susceptibility.

## **Materials and Methods**

**Site description and experimental design and procedures.** This study was conducted as a split plot experiment during the 2010 and 2011 cropping seasons at the University of Nebraska-Lincoln's Mitchell research farm (41°56'N; 103°41'W). Each year, seven sugar beet varieties from three seed breeders (Betaseed®, Crystal®, and Hillebrand®) made up the main plot experimental factor. These varieties had been previously evaluated for SBRA resistance in the laboratory (Hein unpublished data). The sugar beet varieties Beta66RR50, Beta66RR60, HM4093RR, HM9024RR, HM9027RR, and HM9042RR were tested each year with CRR714 also tested in 2010 and Beta37RR22 tested during the 2011 growing season. Seven, four-row plots (whole-plot experimental units) were seeded into two fields subjected to either spring moldboard plow (conventional tillage) or a strip-tillage management (zone tillage). The plots had a between-row spacing of 56 cm and each measured 30.5 meters in length. Zone tillage implies cultivating only a narrow strip where the crop will be planted, and most of the residue from the previous season's crop is left on the soil surface between the rows (Smith 2013). In this study, tillage was not included as a treatment as it was not replicated; therefore, the replicates for both field locations were incorporated into the data. The seeding rate was 6.35 cm between seeds in a row, and manually thinned to an intra-row spacing of 13-15 cm for a final stand of ca. 123,550-140,800 plants per hectare. A John Deere 71 flex planter was used for seeding the regular, untreated seed-containing pellets. Soil type of the plots is Tripp very fine sandy loam soil. The study areas followed corn production during the preceding years. All fields in which the research plots were located were subjected to overhead irrigation practices. No insecticides were applied to

these fields during the study, but two post-emergence glyphosate applications were performed to control weeds.

Each plot was subdivided into four subplots (split-plot experimental units), each measuring 7.62 meters long. Two border rows on each side of the plot were planted to a resistant sugar beet variety (Beta66RR50) to minimize SBRA movement between adjacent plots. Additionally, a 9.14 meter alley was tilled at both ends of each plot to further discourage aphid movement between replications. The four subplots within each plot were assigned to receive four different levels of artificial SBRA infestation (no, low, medium, and high artificial infestation levels). This design was replicated eight times in a randomized complete block.

Greenhouse-reared SBRA colonies were established on sugar beet grown in tree pots (Stuewe & Sons, Inc.®) to augment infestations in the field. The pots measured 10 cm wide by 36 cm high (2.83 liter volume). Five sugar beet seeds were planted in each pot and subsequently thinned to two sugar beets per pot. Three holes (16 cm diameter) were made next to the sugar beets in each pot, into which five mature, apterous aphids were introduced ( $n = 15$  aphids/pot). The pots were incubated in a greenhouse for three weeks at 23° C, after which they were removed for field infestations. The no-infestation subplots received no aphids, the low subplots received a quarter of soil from a tree pot containing SBRA, the medium infestation received half the soil from a tree pot, and the high infestation received a full tree pot of soil. The number of SBRA's contained within each tree pots was  $> 300$ . Artificial aphid infestations were conducted near the center of each subplot. The specified amount of soil from the tree pots was spread evenly on the soil surface on both sides of a metal flashing linking the two pitfall traps in each subplot.

Prior to harvest, a sample of four sugar beets were dug from each subplot to rate the level of SBRA infestation according to an at-harvest root rating scale (range: 0-5) developed by Hutchison and Campbell (1994). SBRA ratings were conducted on 13-14 September (2010), and



20-21 September (2011). Subplots were machine harvested with a two-row sugar beet harvester. A length of 6.4 meters was harvested from the two inside rows of each subplot. Subsequently, two subsamples (ca. 8-10 sugar beets) from each subplot was collected, weighed and sent for quality analyses. The percentage sugar loss to molasses (SLM), percentage sugar content, total sugar per hectare (kg/ha), beet weight (tons/ha), and top weight (grams) were determined for each subplot to evaluate the impact of SBRA on these yield parameters. Top weights were obtained from the four beets rates for SBRA infestation in each subplot. The remaining yield parameters were quantified at Western Sugar's tare lab located in Scottsbluff, Nebraska from the two subsamples collected in each subplot.

**Beneficial epigeal arthropod response to SBRA populations.** Pitfall traps were used to sample epigeal arthropod predators that are likely to encounter SBRA colonies. Pitfall traps are commonly used to sample epigeal arthropods, e.g. carabids, spiders, and rove beetles (e.g. Weibull *et al.* 2003; Eitzinger and Traugott 2011). Two pitfall traps were located in the center half of each subplot. Two traps were placed within each of the two outside rows of the four-row subplot. Each pair of pitfall traps had a sheet of metal flashing installed between them to increase capture efficacy of beneficial arthropods. Each metal flashing measured ca. 165 cm long x 30 cm high, with ca. 15 cm buried below soil level. Pitfall traps were constructed by making a hole in the soil with a golf hole cutter (107 mm diameter), and inserting a section of PVC piping (76 mm diameter and 150 mm high) into each hole to prevent soil from collapsing into the samples during sampling. A small disposable plastic cup (147 ml capacity), containing a mixture (ca. 38 ml) of ethylene glycol and water (in a 1:3 ratio) as a killing and preservation agent, was placed into each trap at the time of their activation. A few drops (ca. 10 ml) of dishwashing liquid was also added to this mixture to reduce the surface tension. A tight-fitting plastic funnel (75 mm diameter on top with a 25 mm diameter at the bottom) was placed on top of each cup to ensure capture of soil arthropods wandering into the traps. Each pitfall trap was subsequently covered with a plastic lid



(250 mm diameter), leaving ca. 10 cm space between the lid and soil surface for arthropods to enter unrestricted. The lids were affixed to a 406.5 mm x 89 mm piece of wood with 127 mm bolts attached to each end which were used to anchor the lid to the soil surface. Pitfall traps were left in the field for the duration of the growing season and capped with a tight-fitting lid when not activated.

The pitfall traps were activated twice per growing season with samples being removed from the field on 7 July 2010 and 2011, and 3 and 4 August 2010 and 2011, respectively. The pitfall traps were left open for five consecutive days after which the contents were collected, washed (with tap water), and stored in 70% ethyl alcohol until the arthropods could be sorted into their respective taxa. Several beneficial epigeal arthropod taxa were enumerated, including three beetle families (Carabidae, Staphylinidae, and Coccinellidae), spiders (Order: Araneae), harvestmen (Order: Opiliones), and centipedes (Class: Chilopoda). These taxa were chosen for their potential importance in agroecosystems as natural enemies of several pest arthropod species, as well as their abundance in agroecosystems (e.g. Weibull *et al.* 2003; Brewer and Elliot 2004; Eitzinger and Traugott 2011). Because of the abundance of ground beetles in our study, and the importance of this group from other agroecosystem studies (Holland and Luff 2000), this group was identified to species level.

**Statistical procedures.** Because the two tillage types were not replicated, the data were combined in such a way that there were eight replications in total (four from the conventional tilled plots, and four from the zone tillage plots). Because of minimal differences between the four differing levels of artificial SBRA infestation, yield and root rating data from the low, medium, and high infested plots were pooled as an ‘infested’ treatment and compared to the uninfested subplots. All yield data, as well as the root ratings for SBRA, were analyzed with a two-way ANOVA implemented in SAS PROC GLIMMIX, version 9.2 (SAS 2008). Significantly different means among the main and split plot effects were separated using a Tukey’s HSD ad

hoc mean comparison test at the  $\alpha = 0.05$  level of significance. Mean comparisons with a marginal significance ( $P \leq 0.07$ ) are also discussed. In addition, Pearson's correlation was calculated to evaluate the relationship between SBRA ratings and the five different yield parameters obtained for all varieties. This was done using the PROC CORR procedure in SAS, version 9.2 (SAS 2008).

For comparing beneficial arthropod activity between the varieties and SBRA infestation levels, the data from the two separate sampling dates within each season were pooled. Statistical analyses followed the same procedure as described for the various yield parameters in the preceding paragraph. For ground beetles, four indices were generated for each variety during each year. These were the cumulative total number of ground beetles, species count (species richness), Simpson's diversity index, and Simpson's measure of evenness. Simpson's diversity index quantifies the diversity within a habitat and is calculated by:

$$D = \sum p_i^2$$

where  $p_i$  is the proportion (from the total count of all species) of individuals for the  $i$ th species (Magurran 2004). Simpson's diversity index accounts for both species richness (i.e. number of species in a sample) as well as evenness (i.e. the relative abundance of each species in the sample).

The reciprocal of Simpson's diversity index was used to calculate the diversity of ground beetles found for each sugar beet variety. The reciprocal index ranges on a scale from one to a maximum equal to the total number of species collected within the sample/habitat. The higher the value of this index is, the more even and diverse the species assemblage of the sample/habitat (Magurran 2004). Simpson's evenness was calculated as:

$$E_{1/D} = (1/D) / S$$

where  $S$  represents the number of species in the sample/habitat. Simpson's evenness ranges on a scale from 0-1 with one indicating complete evenness (i.e. the proportion of each species is equal).

Lastly, ground beetle assemblages between the individual sugar beet varieties were compared by means of the Sorensen's quantitative index (Magurran 2004) as:

$$C_N = 2jN / (N_a + N_b)$$

where  $N_a$  represents the total number of ground beetles caught in sugar beet variety a, and  $N_b$  represents the total caught in sugar beet variety b. Since seven varieties were evaluated each year, pairwise comparisons were made between each of them. The term  $2jN$  represents the sum of the lower number of ground beetles for each species caught in both varieties. Sorensen's quantitative index is a measure of similarity, with higher values indicating lower  $\beta$ -diversity or high similarity (Magurran 2004) on a 0-1 scale.  $\beta$ -diversity, therefore, measures the extent of the difference in diversity between two or more spatial units (Magurran 2004).

## Results

A marginally significant main effect for the level of artificial SBRA infestation (uninfested versus infested) was observed for SBRA ratings during 2010 ( $F_{1,49} = 3.82$ ,  $P = 0.06$ ) and 2011 ( $F_{1,49} = 11.14$ ,  $P = 0.002$ ). Mean ( $\pm$  SEM) SBRA ratings were higher in the artificially infested plots during both years compared to the uninfested plots ( $1.14 \pm 0.12$  vs.  $0.89 \pm 0.13$  during 2010;  $1.35 \pm 0.11$  vs.  $1.05 \pm 0.12$  during 2011), indicating that the artificial infestations helped increase SBRA numbers under the 'infested' treatment. The main effect for variety was also significant for SBRA ratings during both years (Tables 3.1 and 3.2). During both seasons, SBRA infestation was highest in the variety HM4093RR, exceeding a value of 2 on the root rating scale. Furthermore, the varieties HM9027RR (2010 and 2011), CRR714 (2010), and Beta37RR22 (2011) did not differ significantly in their ratings from HM4093RR. Conversely, the

lowest ratings were obtained for Beta66RR50 in 2010 and Beta66RR60 in 2011. The rating for Beta66RR50 in 2010 was not statistically different from those of Beta66RR60, HM9024RR, and HM9042RR, while the rating obtained for Beta66RR60 was not different from that of Beta66RR50 during 2011. The cultivars Beta66RR60 and Beta66RR50 were the only varieties with a mean rating index  $< 1$  during 2011. No interaction between SBRA infestation and variety existed during either season (2010:  $F_{6,49} = 1.44$ ,  $P = 0.22$ , and 2011:  $F_{6,49} = 1.62$ ,  $P = 0.16$ ).

For SLM, the aphid infestation main effect was significant during 2010 ( $F_{1,49} = 22.38$ ,  $P < .001$ ), but not in 2011 ( $F_{1,49} = 0.08$ ,  $P = 0.77$ ). The mean ( $\pm$  SEM) percentage SLM was higher under the infested treatment during 2010 ( $1.17 \pm 0.02$  vs.  $1.10 \pm 0.02$ ). There was a significant difference in SLM between the varieties during both seasons (Tables 3.1 and 3.2). In 2010, the variety CRR714 had a significantly higher SLM compared to HM9042RR. Beta37RR22 had a significantly higher SLM compared to Beta66RR50, HM9024RR and HM9042RR in 2011. For SLM, no interaction between the level of SBRA infestation and variety was observed for either season (2010:  $F_{6,49} = 1.10$ ,  $P = 0.37$ , and 2011:  $F_{6,49} = 0.62$ ,  $P = 0.71$ ).

There were no differences in sugar content between the SBRA infested and uninfested plots during either seasons (2010:  $F_{1,49} = 1.85$ ,  $P = 0.18$ , and 2011:  $F_{1,49} = 0.20$ ,  $P = 0.66$ ). However, the main effect for variety was significant both years (Tables 3.1 and 3.2). The SBRA ratings observed for the different varieties seemed to relate well to differences in sugar content. During 2010, the varieties with the lowest SBRA rating (Beta66RR50 and Beta66RR60) yielded the highest sugar content, while CRR714 and HM4093RR had the lowest sugar content (along with the highest SBRA ratings) (Table 3.1). A similar situation was observed during 2011 when Beta66RR50 and Beta66RR60 again had the highest sugar content, while the most infested varieties (HM4093RR and Beta37RR22) had the lowest sugar content (Table 3.2). During both years, no interactions between the two main effects were observed (2010:  $F_{6,49} = 1.02$ ,  $P = 0.42$ , and 2011:  $F_{6,49} = 0.80$ ,  $P = 0.58$ ).

The level of SBRA infestation did not impact tonnage in either year (2010:  $F_{1,49} = 1.14$ ,  $P = 0.29$ , and 2011:  $F_{1,49} = 0.96$ ,  $P = 0.33$ ). While no differences between varieties for tonnage were observed in 2010 (Table 3.1), there was a difference between the varieties during 2011 (Table 3.2). Root yield was significantly higher for the variety HM4093RR compared to Beta37RR22 during this season. Interestingly, the highest tonnage was measured for HM4093RR which also had the highest SBRA ratings. No interaction between variety and SBRA infestation was observed for either year (2010:  $F_{6,49} = 0.58$ ,  $P = 0.75$ , and 2011:  $F_{6,49} = 0.59$ ,  $P = 0.73$ ).

Sugar yield (kg/ha) was unaffected by SBRA infestations in 2010 and 2011 (2010:  $F_{1,49} = 2.01$ ,  $P = 0.16$ , and 2011:  $F_{1,49} = 1.17$ ,  $P = 0.28$ ). Although there were no statistically significant differences between varieties during 2010 in terms of this yield parameter (Table 3.1), the trend was consistent with those of sugar content during both years. Varietal differences were, however, observed during 2011 (Table 3.2). The variety Beta37RR22 showed the lowest sugar yield during this season. No interaction between the main effects was evident (2010:  $F_{6,49} = 0.77$ ,  $P = 0.60$ , and 2011:  $F_{6,49} = 0.49$ ,  $P = 0.81$ ).

For top weight, the main effect for SBRA infestation was not significant in 2010 ( $F_{1,49} = 1.06$ ,  $P = 0.31$ ) and 2011 ( $F_{1,49} = 0.92$ ,  $P = 0.34$ ). However, there were significant differences in top weight between the varieties during both years (Tables 3.1 and 3.2). In both years, the variety HM4093RR exhibited the lowest top weight, being significantly different from CRR714 and Beta66RR50 in 2010, and from Beta66RR50, Beta37RR22, and Beta66RR60 in 2011. For both years, no interaction between SBRA infestation and varieties were observed (2010:  $F_{6,49} = 0.19$ ,  $P = 0.98$ , and 2011:  $F_{6,49} = 1.21$ ,  $P = 0.32$ ).

SBRA ratings showed a significant negative correlation with sugar yield during both 2010 ( $r = -0.32$ ,  $n = 224$ ,  $P < .001$ ) and 2011 ( $r = -0.25$ ,  $n = 224$ ,  $P < .001$ ). The same was true for SBRA ratings against sugar content in 2010 ( $r = -0.67$ ,  $n = 224$ ,  $P < .001$ ) and 2011 ( $r = -0.65$ ,  $n =$

224,  $P < .001$ ). Finally, a significant negative correlation was also observed between SBRA ratings and top weight during both years (2010:  $r = -0.20$ ,  $n = 224$ ,  $P = 0.003$ , and 2011:  $r = -0.14$ ,  $n = 224$ ,  $P = 0.04$ ). In contrast, SLM did not correlate well with SBRA ratings in either year (2010:  $r = 0.06$ ,  $n = 224$ ,  $P = 0.38$ , and 2011:  $r = 0.009$ ,  $n = 224$ ,  $P = 0.89$ ). Likewise, the correlation between SBRA ratings and tonnage was non-significant for both years (2010:  $r = -0.04$ ,  $n = 224$ ,  $P = 0.60$ , and 2011:  $r = 0.05$ ,  $n = 224$ ,  $P = 0.47$ ).

A total of 16,208 beneficial arthropods were captured during the 2010 field season (Table 3.3). During this season, ground beetles were the most commonly collected taxon. The number of beneficial arthropods caught during the 2011 field season (26,429) was more than 1.6 times higher than the previous season, primarily due to centipede abundance. Coccinellids were not sampled during 2010, but their low numbers ( $n = 31$ ) from the 2011 season resulted in this taxon being excluded from any further analyses.

A two-way ANOVA indicated that the level of SBRA infestation had no impact on centipede (Figure 3.1) and spider (Figure 3.2) activity densities during either 2010 or 2011. Similarly, sugar beet variety did not impact the activity of these two taxa, with no interaction observed between the two main effects (variety and SBRA infestation level). In contrast, the main effect for SBRA infestation was significant for both harvestmen (Figure 3.3) and rove beetles (Figure 3.4) during 2010. For both taxa, activity density was significantly higher in the infested plots during this season. However, this difference did not exist for either harvestmen or rove beetles during the 2011 field season. Sugar beet variety did not impact harvestmen or rove beetle activity, while no interactions between the two main effects were observed for any season. For ground beetles, the results were inconsistent. Ground beetle activity density was significantly higher in the infested treatment during 2010 (Figure 3.5). However, during 2011 significantly more ground beetles were caught in the uninfested treatment. As in the case of the other taxa,

sugar beet variety did not affect ground beetle activity density, with no interactions between the two main effects observed during both years.

This study yielded a total of 12,446 Carabidae, comprised of 66 species in 28 genera (Table 3.4). During the 2010 field season 6,746 carabids were captured, and 5,700 were captured in 2011. The species *Bembidion quadrimaculatum oppositum* Say was the most commonly collected ground beetle during both years (> 30%). Only a few species dominated the total pitfall captures. Nine species in 2010 and eight species in 2011 accounted for ca. 90% of all pitfall captures (Table 3.4). The total species count (species richness) (range: 26-35), Simpson's diversity (range: 5.34-6.08), and Simpson's evenness (range: 0.16-0.22) values for ground beetles collected for each variety are presented in Table 3.5. Consistently low values for Simpson's evenness and Simpson's diversity index were observed for all varieties in both years agreeing with the results that the diversity was dominated by only a few species. Furthermore, the pairwise comparisons of  $\beta$ -diversity (Sorensen's quantitative index) throughout the study period (Tables 3.6 and 3.7) showed that no Sorensen's quantitative index value was lower than 0.85, signifying a high degree of similarity in the ground beetle  $\beta$ -diversity between all varieties tested.

### **Discussion and Conclusions**

Varietal resistance towards insect pests remains a cornerstone in insect pest management programs (Niranjan and Khush 1995; Van Lenteren *et al.* 1995). The importance of this technology is magnified for insect pests, such as the SBRA, for which chemical control options are currently unavailable. Winter and Patrick (1997) reported complete crop loss of sugar beets in the Texas Panhandle when susceptible cultivars were grown under high SBRA pressure. With the exception of the percentage SLM in 2010, the scale of the differences between the SBRA uninfested and infested treatments appeared to have been insufficient to discern a measurable impact on the remaining yield parameters, even though there were significantly higher SBRA

ratings under the infested treatments (for both years). Despite the uninfested plots receiving no artificial SBRA infestations, they were still subjected to natural infestations from colonizing aphids moving into the plots and were not devoid of SBRA infestation. Therefore, it can be concluded that to truly test for the impact of the differing SBRA infestation levels on these crop parameters, it would be necessary to compare sugar beet roots kept clean of SBRA with those of infested beets. However, because field plantings are subjected to natural aphid infestations, and because no chemicals are registered for SBRA control, this practice was not feasible in this particular study.

The results from this study indicate that some of the glyphosate-tolerant sugar beet varieties tested have a high level of resistance against the SBRA under field conditions in western Nebraska. This was clearly observed for the varieties Beta66RR50 and Beta66RR60, with SBRA root rating of  $< 0.50$  during both years (Tables 3.1 and 3.2). However, the cultivars HM4093RR, HM9027RR, CRR714, and Beta37RR22 were susceptible to SBRA infestations. From their studies in Minnesota, Hutchison and Campbell (1994) reported that SBRA ratings should exceed a value of two (dependent on precipitation levels) on the root rating scale for consistent losses in recoverable sugar to occur. Although HM4093RR was the only variety to exceed this value consistently, the remaining susceptible varieties had ratings approaching this value ( $> 1.75$  in all cases).

In addition to SBRA rating differences amongst the varieties, there were also several differences between the varieties in terms of the various yield parameters evaluated. Three of these parameters (sugar content, yield, and top weight) showed a significant negative correlation in both years with SBRA ratings. Therefore, for these three parameters at least, a portion of the observed variability between varieties can be ascribed to the impact of SBRA, while genetic differences between the varieties could also have contributed. In light of the non-significant correlations obtained between SBRA ratings with tonnage and SLM, the observed varietal



differences were most likely caused by differences in the genetic background between these varieties, rather than SBRA pressure. As an example, HM9043RR yielded the highest tonnage, despite having the highest SBRA rating.

Differences in sugar content and yield (kg/ha) between varieties reflected the differences in SBRA root rating between the varieties. This was further supported by the significant negative correlations obtained for these two yield parameters, measured against root aphid ratings. A similar situation was also seen by Harper (1963), Hutchison and Campbell (1994) and Winter (1999) who saw a decreased sugar content in heavily infested sugar beets. However, these findings are in contrast with Summers and Newton (1989) who observed little impact of root aphids on sugar content in California. Possible explanations for the conflicting results put forth here may be due to differing cultivation practices, irrigation methods and frequency, geographical differences between production regions, or even due to different aphid biotypes or species. There is still much controversy over the true identity of *Pemphigus betae* in sugar beets (Harper 1963; Footitt *et al.* 2010) – i.e., *P. betae* may be a polyphyletic group. Winter and Patrick (1997) reported that severely infested sugar beets showed visible signs of top wilting three months prior to harvest, explaining some of the differences in top weight observed between varieties in this study.

Pest resistant varieties lend themselves perfectly towards integration with other management tactics, such as conservation biological control which relies on the encouragement of natural enemies already present within the crop (Speight *et al.* 2008). The high number of epigeal beneficial arthropods trapped in this study demonstrates the potential of these organisms to contribute to conservation biological control in sugar beet agroecosystems. However, the results from this study have shown that the activity of these organisms fluctuate between seasons and/or localities, as can be seen with the difference in the total number of epigeal arthropods collected between the two years. Chang and Kareiva (1999) relate these inter-seasonal variances

in natural enemy abundance within agroecosystems to abiotic influences and farming practices (e.g. cultivation). With the possible exception of rove beetles, this was well exemplified by the numbers of the remaining predatory groups (e.g. centipedes).

The effect of generalist natural enemies (whether single species or a complex of species) on pest populations can be significant, and developing management strategies and techniques to conserve the function of natural enemies present within the habitat has a growing interest (Symondson *et al.* 2002a). Despite the higher activity densities observed for harvestmen, rove beetles, and ground beetles in the artificially infested SBRA treatments during 2010, it is difficult to relate this phenomenon to the higher SBRA infestation levels measured in this treatment. This is because the same pattern was not evident during the 2011 field season where ground beetle activity density was actually higher under the uninfested treatment. In addition, it would have been expected that more predators should have been observed in the treatments containing the SBRA susceptible varieties which supported higher SBRA population densities. However, no such relationship was apparent, because variety had no effect on any of the beneficial taxa's activity densities. Furthermore, ground beetle numbers were not highest in the plots containing the highest SBRA-infested varieties (Table 3.5).

These results do not support a numerical response of beneficial arthropods towards SBRA infestations, but this does not indicate that the observed beneficial arthropods make no contribution towards SBRA control. This is a common situation observed for generalist natural enemies (Symondson *et al.* 2002a). It is known, for example, that generalist predators are capable of preventing aphid outbreaks, rather than numerically responding to these outbreaks, as is often the case with aphid-specific predators (Edwards *et al.* 1979). Lastly, Symondson *et al.* (2002b) found it unlikely that generalist predators will respond numerically or aggregate in response to single prey species. The exact contribution of these generalist epigeal predators to SBRA management will only become apparent under more controlled predation studies where both

aphid and predator numbers can be manipulated and directly observed, or through the use of serological techniques, such as enzyme-linked immunosorbent assays (ELISA) (Symondson *et al.* 2002b; Speight *et al.* 2008). Indeed, with observational experiments such as this, it is difficult to determine the contribution of generalist predatory natural enemies on prey populations (Potts and Vickerman 1974); however, these results do identify the potential predatory taxa that warrant further investigation (Speight *et al.* 2008). One such highly-speciose group identified from this study was ground beetles.

Ground beetles are effective generalist natural enemies of invertebrate pests (Sunderland 2001). They are also known to feed on the seeds of many species of weeds, thereby contributing to sustainable weed management (Honek *et al.* 2003). Ground beetles are known to be abundant in agroecosystems (Holland and Luff 2000), a view supported by this study. They are important predators of soil inhabiting pests (Griffiths *et al.* 1985), highlighting their importance as potential predators of the SBRA. This study recorded a large number of ground beetle species of which only a limited number dominated the total pitfall captures. However, this phenomenon is typical of agricultural settings (Hance *et al.* 1990; Fan *et al.* 1993; Ellsbury *et al.* 1998; Holland and Luff 2000; Luff 2002; O'Rourke *et al.* 2008). Ground beetle species assemblages are known to be variable between different crop types, cropping systems, tillage practices, and geographical location (Holland and Luff 2000). This study showed that the species count, activity density, diversity, and evenness varied little between the different sugar beet varieties (further supported by Sorensen's quantitative index). This may have been due to the similarity in the crop phenology and the associated husbandry practices leading to a uniform microclimate within the crop. In addition, the relatively small size of the plots may have resulted in ground beetle movement between the plots. Even though the two tillage practices were not directly comparable in this study, it was observed that the zone tillage system supported higher activity densities of carabids. This aspect is investigated further in Chapter 5.

In conclusion, genetic resistance towards SBRA in sugar beet does significantly suppress aphids in western Nebraska; contributing to increases in the quality and quantity of sugar produced. An abundance of epigeal natural enemies were captured with pitfall trapping during this study. However, being mostly generalist predators, there was no direct association with high SBRA populations in susceptible sugar beet varieties harboring high aphid populations. Nonetheless, ground beetles were identified as an abundant component of the natural enemy fauna, warranting further investigation into their functional roles within sugar beet agroecosystems, and their contribution to SBRA management. In addition, many ground beetle species also feed on weed seeds, further contributing to their importance in agroecosystems.

**Table 3.1:** Sugar beet varietal differences ( $n = 7$  varieties) for sugar beet root aphid (SBRA) rating and five selected yield parameters as determined by a two-way ANOVA for the 2010 growing season.

Variable	Beta66RR60	HM4093RR	Beta66RR50	HM9024RR	CRR714
SBRA rating	$0.48 \pm 0.24^a$	$2.10 \pm 0.24^b$	$0.01 \pm 0.24^a$	$0.49 \pm 0.24^a$	$1.92 \pm 0.24^b$
SLM	$1.17 \pm 0.04^{a,b}$	$1.13 \pm 0.04^{a,b}$	$1.19 \pm 0.04^{a,b}$	$1.08 \pm 0.04^{a,b}$	$1.23 \pm 0.04^a$
Sugar content	$18.99 \pm 0.34^{a,b}$	$17.45 \pm 0.34^{c,d}$	$19.30 \pm 0.34^a$	$18.65 \pm 0.34^{a,b,c}$	$16.66 \pm 0.34^d$
Tons/ha	$56.99 \pm 3.11^a$	$57.46 \pm 3.11^a$	$54.43 \pm 3.11^a$	$53.31 \pm 3.11^a$	$56.86 \pm 3.11^a$
kg sugar/ha	$10829 \pm 513^a$	$9997 \pm 513^a$	$10489 \pm 513^a$	$9768 \pm 513^a$	$9510 \pm 513^a$
Top weight (g)	$209.25 \pm 12.36^{a,b}$	$166.72 \pm 12.36^b$	$232.49 \pm 12.36^a$	$210.98 \pm 12.36^{a,b}$	$232.6 \pm 12.36^a$

**Table 3.1:** Continued.

Variable	HM9042RR	HM9027RR	df	<i>F</i>	<i>P</i>
SBRA rating	$0.35 \pm 0.24^a$	$1.77 \pm 0.24^b$	6, 42	14.19	< .001
SLM	$1.02 \pm 0.04^b$	$1.12 \pm 0.04^{a,b}$	6, 42	2.99	0.02
Sugar content	$18.60 \pm 0.34^{a,b,c}$	$17.85 \pm 0.34^{b,c,d}$	6, 42	9.08	< .001
Tons/ha	$52.55 \pm 3.11^a$	$56.59 \pm 3.11^a$	6, 42	0.92	0.49
kg sugar/ha	$9768 \pm 513^a$	$10109 \pm 513^a$	6, 42	1.12	0.37
Top weight (g)	$186.82 \pm 12.36^{a,b}$	$206.29 \pm 12.36^{a,b}$	6, 42	4.79	0.001

SBRA = Sugar Beet Root Aphid, SLM = Sugar Loss to Molasses. Means ( $\pm$  SEM) followed by different letters in a row are significantly different at  $\alpha = 0.05$  as determined by a post-hoc Tukey mean comparison test.

**Table 3.2:** Sugar beet varietal differences ( $n = 7$  varieties) for sugar beet root aphid (SBRA) rating and five selected yield parameters as determined by a two-way ANOVA for the 2011 growing season.

Variable	Beta66RR60	HM4093RR	Beta66RR50	HM9024RR	Beta37RR22
SBRA rating	$0.07 \pm 0.17^a$	$2.15 \pm 0.17^d$	$0.18 \pm 0.17^a$	$1.12 \pm 0.17^b$	$1.91 \pm 0.17^d$
SLM	$1.51 \pm 0.07^{a,b}$	$1.55 \pm 0.07^{a,b}$	$1.47 \pm 0.07^b$	$1.39 \pm 0.07^b$	$1.72 \pm 0.07^a$
Sugar content	$17.07 \pm 0.23^a$	$13.79 \pm 0.23^{d,e}$	$16.50 \pm 0.23^a$	$14.88 \pm 0.23^{b,c}$	$13.11 \pm 0.23^e$
Tons/ha	$50.11 \pm 3.20^{a,b}$	$52.64 \pm 3.20^a$	$46.90 \pm 3.20^{a,b}$	$50.89 \pm 3.20^{a,b}$	$42.69 \pm 3.20^b$
kg sugar/ha	$8556 \pm 459^a$	$7252 \pm 459^{a,b}$	$7774 \pm 459^{a,b}$	$7488 \pm 459^{a,b}$	$5640 \pm 459^c$
Top weight (g)	$260.26 \pm 12.25^a$	$204.33 \pm 12.25^b$	$273.57 \pm 12.25^a$	$229.96 \pm 12.25^{a,b}$	$267.94 \pm 12.25^a$

**Table 3.2:** Continued.

Variable	HM9042RR	HM9027RR	df	<i>F</i>	<i>P</i>
SBRA rating	$1.20 \pm 0.17^{b,c}$	$1.79 \pm 0.17^{c,d}$	6, 42	35.08	< .001
SLM	$1.41 \pm 0.07^b$	$1.54 \pm 0.07^{a,b}$	6, 42	4.28	0.002
Sugar content	$15.43 \pm 0.23^b$	$14.04 \pm 0.23^{c,d}$	6, 42	49.52	< .001
Tons/ha	$48.05 \pm 3.20^{a,b}$	$48.10 \pm 3.20^{a,b}$	6, 42	2.63	0.03
kg sugar/ha	$7412 \pm 459^{a,b}$	$6722 \pm 459^{b,c}$	6, 42	9.22	< .001
Top weight (g)	$247.18 \pm 12.25^{a,b}$	$236.87 \pm 12.25^{a,b}$	6, 42	4.23	0.002

SBRA = Sugar Beet Root Aphid, SLM = Sugar Loss to Molasses. Means ( $\pm$  SEM) followed by different letters in a row are significantly different at  $\alpha = 0.05$  as determined by a post-hoc Tukey mean comparison test.

**Table 3.3:** Total number of beneficial arthropods (by taxon) collected with pitfall trapping during 2010 and 2011.

Beneficial arthropod taxon	Total number collected	
	2010 <sup>a</sup>	2011 <sup>a</sup>
Araneae (spiders)	1,996	1,458
Carabidae (ground beetles)	6,746	5,700
Chilopoda (centipedes)	4,630	15,518
Coccinellidae (lady beetles)	-	31
Opiliones (harvestmen)	1,199	2,089
Staphylinidae (rove beetles)	1,637	1,633
Total	16,208	26,429

<sup>a</sup>Total collected over two sampling dates within the specified year ( $n = 896$  pitfall samples/year).

**Table 3.4:** Percentage abundance of ground beetle (Coleoptera: Carabidae) species collected during 2010 and 2011.

Species	% Total	
	2010 <sup>a</sup>	2011 <sup>b</sup>
<i>Agonoleptus conjunctus</i> (Say)	-	0.02
<i>Agonum corvus</i> (LeConte)	-	0.02
<i>Agonum cupreum</i> Dejean	-	0.02
<i>Agonum decorum</i> (Say)	0.03	0.11
<i>Agonum extensicolle</i> (Say)	0.03	0.02
<i>Agonum placidum</i> (Say)	7.59*	1.54
<i>Amara apricaria</i> (Paykull)	0.03	-
<i>Amara carinata</i> (LeConte)	1.94	6.51*
<i>Amara farcta</i> LeConte	0.04	0.88
<i>Amara littoralis</i> Dejean	0.04	0.54
<i>Amara obesa</i> (Say)	0.06	0.26
<i>Amara quenseli quenseli</i> (Schönherr)	0.31	0.09
<i>Bembidion</i> Latreille sp.	-	0.04
<i>Bembidion impotens</i> Casey	0.01	-
<i>Bembidion nitidum</i> (Kirby)	0.31	0.18
<i>Bembidion obscurellum obscurellum</i> (Motschulsky)	3.99*	7.60*
<i>Bembidion quadrimaculatum oppositum</i> Say	35.72*	30.23*
<i>Bembidion rapidum</i> (LeConte)	3.91*	0.18
<i>Bembidion tetracolum tetracolum</i> Say	15.30*	8.68*
<i>Bembidion versicolor</i> (LeConte)	-	0.02
<i>Bradycellus congener</i> (LeConte)	0.01	-
<i>Calosoma affine</i> Chaudoir	0.07	0.04
<i>Chlaenius pennsylvanicus pennsylvanicus</i> Say	-	0.04
<i>Chlaenius sericeus</i> (Forster)	0.03	0.02
<i>Chlaenius tomentosus</i> (Say)	0.01	0.07
<i>Chlaenius tricolor tricolor</i> Dejean	0.07	0.33
<i>Cicindela punctulata punctulata</i> Olivier	2.67*	4.16*
<i>Cicindela terricola cinctipennis</i>	0.06	-
<i>Clivina</i> Latreille sp.	0.01	0.19
<i>Clivina impressifrons</i> LeConte	0.03	-
<i>Cratacanthus dubius</i> (Palisot de Beauvois)	0.01	0.02
<i>Cyclotrachelus heros</i> (Say)	-	0.02
<i>Cyclotrachelus torvus</i> (LeConte)	0.55	0.07
<i>Diplocheila</i> Brullé sp.	0.01	-
<i>Discoderus parallelus</i> (Haldeman)	0.01	0.02
<i>Dyschirius</i> Bonelli sp.	-	0.02
<i>Dyschirius globulosus</i> (Say)	0.34	-



<i>Elaphropus anceps</i> (LeConte)	7.81*	24.40*
<i>Euryderus grossus</i> (Say)	0.22	0.07
<i>Galerita janus</i> (Fabricius)	0.01	-
<i>Harpalus amputatus amputatus</i> Say	1.93	1.35
<i>Harpalus caliginosus</i> (Fabricius)	0.07	0.12
<i>Harpalus desertus</i> LeConte	0.15	-
<i>Harpalus erraticus</i> Say	0.64	0.46
<i>Harpalus fraternus</i> LeConte	0.09	-
<i>Harpalus fuscipalpis</i> Sturm	0.06	0.02
<i>Harpalus herbivagus</i> Say	0.18	0.11
<i>Harpalus pensylvanicus</i> (DeGeer)	2.91*	1.89*
<i>Harpalus reversus</i> Casey	0.19	0.25
<i>Harpalus somnulentus</i> Dejean	0.09	-
<i>Loricera pilicornis</i> (Fabricius)	1.11	0.11
<i>Microlestes linearis</i> (LeConte)	0.64	0.54
<i>Paraclivina ferrea</i> (LeConte)	-	0.93
<i>Pasimachus elongatus</i> LeConte	-	0.04
<i>Piosoma setosum</i> LeConte	-	0.02
<i>Poecilus chalcites</i> (Say)	0.01	0.07
<i>Poecilus lucublandus</i> (Say)	0.34	1.05
<i>Poecilus scitulus</i> LeConte	0.64	0.60
<i>Pterostichus femoralis</i> (Kirby)	0.03	0.07
<i>Pterostichus permundus</i> (Say)	0.03	0.07
<i>Scarites subterraneus</i> Fabricius	-	0.04
<i>Scarites vicinus</i> Chaudoir	0.01	-
<i>Selenophorus planipennis</i> LeConte	0.01	0.02
<i>Stenolophus comma</i> (Fabricius)	9.43*	5.89*
<i>Stenolophus conjunctus</i> Say	0.04	-
<i>Stenolophus lineola</i> (Fabricius)	0.10	-
Sum	100	100
Number of species	54	52

<sup>a</sup> A total of 6,746 ground beetles collected during two sampling dates.

<sup>b</sup> A total of 5,700 ground beetles collected during two sampling dates.

\* Ground beetle species making up ca. 90% of the total captures within a specified year.

**Table 3.5:** Total number of individuals, species count, Simpson's diversity, and Simpson's evenness for ground beetles (Coleoptera: Carabidae) collected in the different glyphosate-tolerant sugar beet varieties during 2010 and 2011.

Variety	Total number of individuals <sup>a</sup>	Species count <sup>a</sup>	Simpson's diversity <sup>a</sup>	Simpson's evenness <sup>a</sup>
2010				
Beta66RR60	852	33	5.35	0.16
HM4093RR	1,039	33	5.54	0.17
Beta66RR50	1,021	34	5.34	0.16
HM9024RR	1,061	33	5.65	0.17
CRR714	851	26	5.59	0.22
HM9042RR	925	30	5.81	0.19
MH9027RR	997	35	5.94	0.17
2011				
Beta66RR60	876	30	5.55	0.18
HM4093RR	783	29	5.50	0.19
Beta66RR50	808	29	5.39	0.19
HM9024RR	807	34	5.59	0.16
Beta37RR22	771	30	6.08	0.20
HM9042RR	842	30	5.79	0.19
MH9027RR	813	34	5.95	0.18

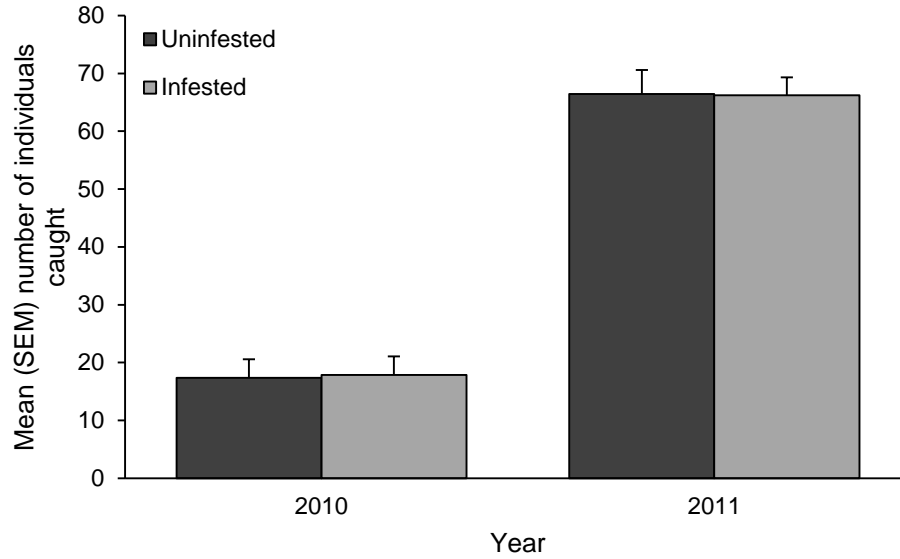
<sup>a</sup> Data represent the cumulative total of ground beetles collected within a year and within a particular variety over eight replications and two sampling dates.

**Table 3.6:** Pairwise comparisons (Sorensen's quantitative index) of ground beetle (Coleoptera: Carabidae) abundance and species richness between different glyphosate-tolerant sugar beet varieties to yield a measure of the  $\beta$ -diversity during the 2010 cropping season.

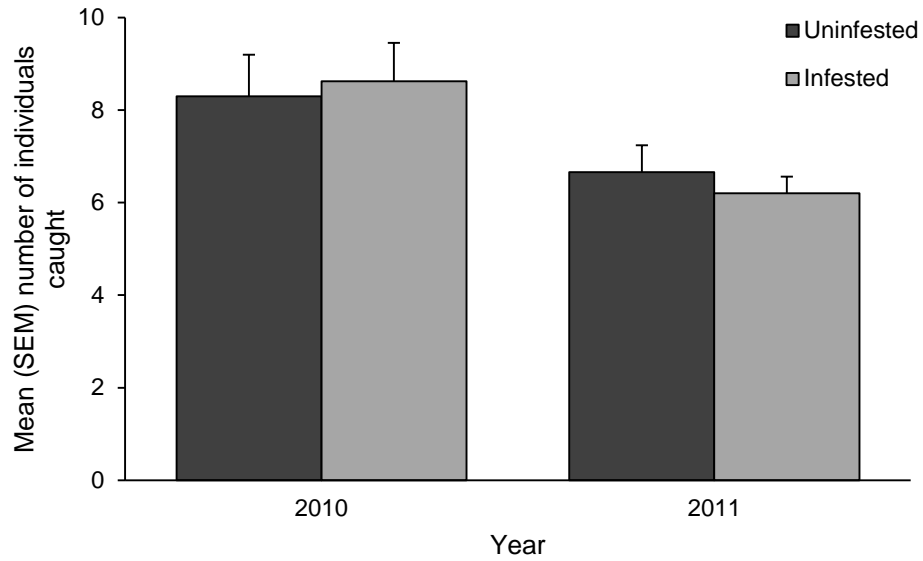
	HM4093RR	Beta66RR50	HM9024RR	CRR714	HM9042RR	HM9027RR
Beta66RR60	0.87	0.86	0.85	0.91	0.92	0.88
HM4093RR		0.92	0.92	0.88	0.91	0.92
Beta66RR50			0.91	0.88	0.88	0.91
HM9024RR				0.86	0.87	0.91
CRR714					0.89	0.90
HM9042RR						0.92

**Table 3.7:** Pairwise comparisons (Sorensen's quantitative index) of ground beetle (Coleoptera: Carabidae) abundance and species richness between different glyphosate-tolerant sugar beet varieties to yield a measure of the  $\beta$ -diversity during the 2011 cropping season.

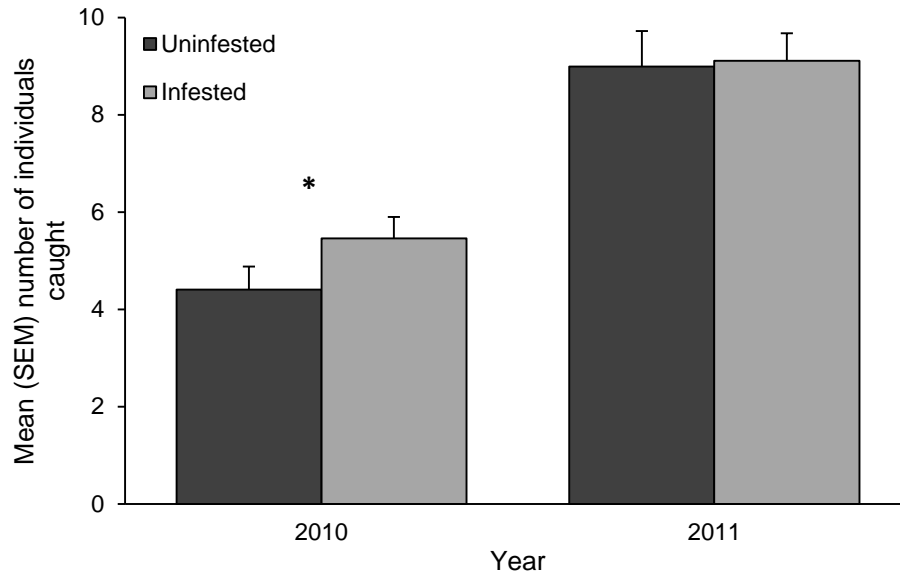
	HM4093RR	Beta66RR50	HM9024RR	Beta37RR22	HM9042RR	HM9027RR
Beta66RR60	0.90	0.90	0.91	0.89	0.92	0.90
HM4093RR		0.92	0.94	0.90	0.91	0.90
Beta66RR50			0.92	0.87	0.91	0.90
HM9024RR				0.88	0.92	0.92
Beta37RR22					0.88	0.90
HM9042RR						0.91



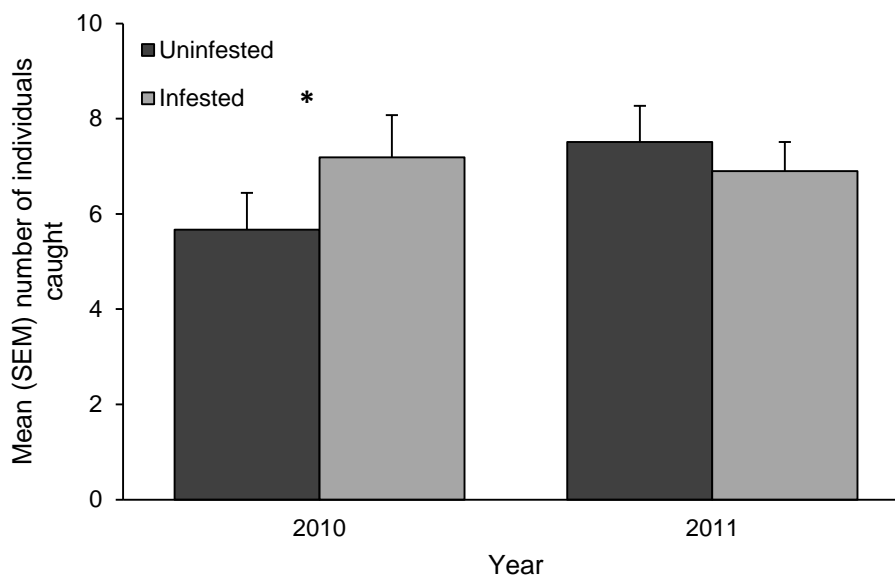
**Figure 3.1:** Mean ( $\pm$  SEM) number of centipedes (Class: Chilopoda) collected during the 2010 and 2011 field seasons in unfested and infested plots. Results for a two-way ANOVA: variety:  $F_{6,42} = 0.89$ ,  $P = 0.51$ ; infestation:  $F_{1,49} = 0.16$ ,  $P = 0.69$ ; variety x infestation:  $F_{6,49} = 0.50$ ,  $P = 0.81$ , and variety:  $F_{6,42} = 0.53$ ,  $P = 0.78$ ; infestation:  $F_{1,49} = 0$ ,  $P = 0.96$ ; variety x infestation:  $F_{6,49} = 1.04$ ,  $P = 0.41$ , during 2010 and 2011, respectively.



**Figure 3.2:** Mean ( $\pm$  SEM) number of spiders (Order: Araneae) collected during the 2010 and 2011 field seasons in unfested and infested plots. Results for a two-way ANOVA: variety:  $F_{6,42} = 1.08$ ,  $P = 0.39$ ; infestation:  $F_{1,49} = 0.26$ ,  $P = 0.61$ ; variety x infestation:  $F_{6,49} = 1.07$ ,  $P = 0.39$ , and variety:  $F_{6,42} = 0.09$ ,  $P = 1.00$ ; infestation:  $F_{1,49} = 0.57$ ,  $P = 0.46$ ; variety x infestation:  $F_{6,49} = 0.33$ ,  $P = 0.92$  during 2010 and 2011, respectively.

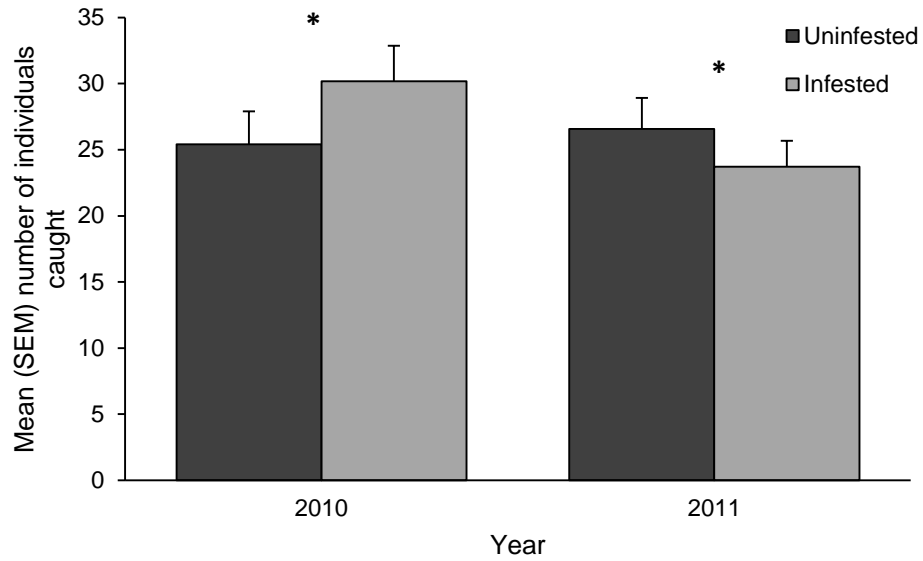


**Figure 3.3:** Mean ( $\pm$  SEM) number of harvestmen (Order: Opiliones) collected during the 2010 and 2011 field seasons in unfested and infested plots. Results for a two-way ANOVA: variety:  $F_{6,42} = 1.38$ ,  $P = 0.24$ ; infestation:  $F_{1,49} = 5.21$ ,  $P = 0.03$ ; variety x infestation:  $F_{6,49} = 0.24$ ,  $P = 0.96$ , and variety:  $F_{6,42} = 0.47$ ,  $P = 0.82$ ; infestation:  $F_{1,49} = 0.03$ ,  $P = 0.85$ ; variety x infestation:  $F_{6,49} = 0.95$ ,  $P = 0.47$ , during 2010 and 2011, respectively. An asterisk indicates a significant difference ( $P < 0.05$ ) between the unfested and infested treatments within that year (Tukey mean comparison test).



**Figure 3.4:** Mean ( $\pm$  SEM) number of rove beetles (Coleoptera: Staphylinidae) collected during the 2010 and 2011 field seasons in unfested and infested plots. Results for a two-way ANOVA: variety:  $F_{6,42} = 0.42$ ,  $P = 0.86$ ; infestation:  $F_{1,49} = 7.24$ ,  $P = 0.01$ ; variety x infestation:  $F_{6,49} = 0.46$ ,  $P = 0.83$ , and variety:  $F_{6,42} = 0.73$ ,  $P = 0.63$ ; infestation:  $F_{1,49} = 1.42$ ,  $P = 0.24$ ; variety x infestation:  $F_{6,49} = 1.61$ ,  $P = 0.16$ , during 2010 and 2011, respectively. An asterisk indicates a significant difference ( $P < 0.05$ ) between the unfested and infested treatments within that year (Tukey mean comparison test).





**Figure 3.5:** Mean ( $\pm$  SEM) number of ground beetles (Coleoptera: Carabidae) collected during the 2010 and 2011 field seasons in unfested and infested plots. Results for a two-way ANOVA: variety:  $F_{6,42} = 1.19$ ,  $P = 0.33$ ; infestation:  $F_{1,49} = 7.15$ ,  $P = 0.01$ ; variety x infestation:  $F_{6,49} = 1.20$ ,  $P = 0.32$ , and variety:  $F_{6,42} = 0.40$ ,  $P = 0.87$ ; infestation:  $F_{1,49} = 4.29$ ,  $P = 0.04$ ; variety x infestation:  $F_{6,49} = 0.41$ ,  $P = 0.87$ , during 2010 and 2011, respectively. An asterisk indicates a significant difference ( $P < 0.05$ ) between the unfested and infested treatments within that year (Tukey mean comparison test).

## CHAPTER 4

### RESPONSE OF SUGAR BEET ROOT APHIDS (*PEMPHIGUS BETAE* DOANE) AND BENEFICIAL EPIGEAL ARTHROPOD COMMUNITIES TO SUGAR BEET PLANT DENSITY AND SEED-APPLIED INSECTICIDE IN WESTERN NEBRASKA

#### Introduction

The sugar beet root aphid, *Pemphigus betae* Doane, is a serious pest of cultivated sugar beet in North America (Hein *et al.* 2009), reducing both sugar and root yield (Summers and Newton 1989; Hutchison and Campbell 1994; Winter 1999; Hein *et al.* 2009). Unfortunately, the subterranean existence of the damaging summer populations complicates conventional chemical control measures (Winter and Patrick 1997; Dewar 2007). Sugar beet root aphids (SBRA) are protected from direct contact with foliar insecticides and, with the exception of certain compounds (Jacobson and Thriugnanam 1991), most systemic compounds are not systemic within phloem to move to the host's roots for adequate control (Dewar and Cooke 2006; Dewar 2007). In the absence of reliable means for aphid control, more emphasis has been placed on an integrated approach to SBRA management including sanitation, irrigation scheduling, crop rotation, control by natural enemies, and the use of resistant varieties (Summers and Newton 1989; Hein *et al.* 2009).

The delivery of plant protectants are increasingly being done through seed application (Halmer 2000). Neonicotinoids comprise a class of insecticides that has become very important in crop protection (Elbert *et al.* 2008; Seagraves and Lundgren 2011; Goulson 2013). Neonicotinoid seed treatments possess lasting residual and systemic effects, and a broad-spectrum activity towards several feeding guilds, rendering them suitable for control of early-season pests (Elbert *et*

*al.* 2008). They are applied to seeds as a film coating, multilayer coating, or seed dressing to protect young plants against arthropod pests, and are used on various crops, including sugar beet (Elbert *et al.* 2008).

Neonicotinoid seed treatments are renowned for their control of sugar beet sucking insect pests and the viruses they transmit on account of their systemic action (Dewar and Read 1990; Schmeer *et al.* 1990; Rouchaud *et al.* 1994; Wauters and Dewar 1996; May 2001; Dewar *et al.* 2002; Dewar and Cooke 2006; Elbert *et al.* 2008; Strausbaugh *et al.* 2010). Thiamethoxam, imidacloprid, and clothianidin are primarily used as seed treatments. Little work has been published on the effect of neonicotinoid seed treatments on SBRA. However, Dewar and Cooke (2006) indicate that seed treatments might be ineffective against root aphids, because root colonization takes place later in the season when the effect of the chemical has dissipated. Furthermore, Westwood *et al.* (1998) found that imidacloprid concentrations remained low in sugar beet roots at different post-planting sampling intervals. However, a recent study suggested that, in addition to good control of the foliar bean aphid (*Aphis fabae* Scopoli), neonicotinoid seed treatments also suppressed *P. betae* incidence (Strausbaugh *et al.* 2010), but perhaps not to levels that can be considered as adequate control.

Beneficial arthropods are susceptible to insecticides (Ellsbury *et al.* 1998), and in many cases, even more so than the target pest (Ruberson *et al.* 1998) due to factors such as smaller body size, with a greater surface to volume ratio, and the presence of lower levels of detoxification enzymes (Hoddle and Van Driesche 2009). However, the conservation of natural enemies of pest arthropods in agroecosystems can be key to an IPM program, preventing some injurious insects from attaining pest status and reducing the damage potential of important pests (Pedigo and Rice 2009). Compared to foliar applications, neonicotinoid seed treatments are often regarded as safer to the environment due to decreased amount of active ingredient, lower mammalian toxicity, and reduced insecticidal contact for non-target organisms, thus providing an

incentive for their use in IPM systems (Mizell and Sconyers 1992; Taylor *et al.* 2001; Albajes *et al.* 2003; Elbert *et al.* 2008; Jeschke and Nauen 2008). However, this should not imply that non-target organisms (such as natural enemies) would remain unaffected by seed-applied insecticides. Natural enemies can be exposed to the chemical when they supplement their diet by feeding on treated plant material (Albajes *et al.* 2003). Several studies conducted under laboratory conditions have found that neonicotinoid seed treatments can have a direct adverse effect on beneficial arthropods by causing mortality (Al-Deeb *et al.* 2001; Mullin *et al.* 2005; Moser and Obrycki 2009; Seagraves and Lundgren 2011). However, studies of seed treatments and natural enemies under field conditions have shown inconsistent results. For example, Seagraves and Lundgren (2011) reported reduced abundance of nabid bugs in thiamethoxam seed-treated soybeans, as well as reduced adult lacewing abundance in imidacloprid seed-treated soybeans. In contrast, Krauter *et al.* (2001) did not measure any negative impact of imidacloprid seed treatments on nabid bugs, geocorid bugs, ladybeetles in the genus *Scymnus* (Pullus), spiders, or lacewings under field conditions. Albajes *et al.* (2003) reported that spiders, ladybeetles, and rove beetles were not impacted negatively by imidacloprid seed-treated corn, while ground beetles were only moderately affected during one of the five years in which the study was conducted. These authors did, however, report a significant negative effect of this insecticide on Heteroptera. Leslie *et al.* (2009) saw decreased abundance for two species of ground beetles in neonicotinoid seed-treated corn plantings. Naveed *et al.* (2010) reported reduced rates of parasitism of *Bemisia tabaci* (Gennadius) in cotton treated with seed-applied thiamethoxam and imidacloprid. Therefore, it is prudent to evaluate the impact of seed-applied insecticides on natural enemies for all cropping systems and natural enemy assemblages in which they are used.

Apart from direct toxicity, early-season prey suppression, due to seed-applied insecticides, could prevent buildup of natural enemies and lessen their impact on later-season pests such as the SBRA. Additionally, natural enemies might also acquire the systemic

insecticides indirectly from their prey as shown by Grafton-Cardwell and Gu (2003) who observed increased toxicity in the larvae of vedalia beetles (*Rodolia cardinalis* (Mulsant)) preying on cottony cushion scale (*Icerya purchasi* (Williston)) that had fed on treated plant material. Papachristos and Milonas (2008) also measured reduced larval survival, reduced adult longevity, and reduced fecundity of a ladybeetle (*Hippodamia undecimnotata* (Schneider)) after feeding on *Aphis fabae* Scopoli which ingested systemic imidacloprid. Because of these potential effects, seed-applied insecticide could pose a particular threat to edaphic natural enemy communities — a group which is very likely to interact with SBRA.

Apart from insecticides, other factors relating to the physical cropping environment are also known to influence both pest and natural enemy population dynamics. With ground beetles, for example, edaphic factors (e.g. soil moisture, soil type, etc.), and crop type, have been reported to affect beetle assemblages (Holland and Luff 2000). However, little information is available on how differences in the cropping environment, such as plant spacing, affect natural enemy and pest species dynamics. For example, Honek (1988) found differing activity of ground beetles depending on vegetation density. In sugar beet, optimal plant spacing is needed to maximize sugar yield (Jaggard and Qi 2006; Smith *et al.* 2013). However, optimal plant establishment with sugar beet is challenged by seed depth, seed placement, soil crusting, soil temperature, soil moisture, blowing soils, seed quality, damage from diseases, and insect pests and pesticides (Smith *et al.* 2013; Yonts *et al.* 2013). On average, sugar beet emergence in Colorado, Wyoming, and Nebraska is estimated at 65%, with fluctuations between 45 to 80% (Smith *et al.* 2002), or 55 to 75% (Smith *et al.* 2013). With such unpredictability, it is essential to understand the responses of pests and their natural enemies to these changes in order to predict if pest pressure, as well as the ecosystem services provided by natural enemies, will be impacted.

The objectives of this study were to evaluate the response of SBRA, resident epigeal beneficial arthropods, and several crop yield parameters to sugar beets seeded with seed-applied

insecticides (Poncho Beta) at varying plant densities under field conditions in western Nebraska. It is hypothesized that plant density, through altering within-row plant spacing, would not affect natural enemy activity density. It is also hypothesized that seed-applied insecticide will affect natural enemy density through direct toxicity or by reducing prey availability.

### **Materials and Methods**

This study was conducted during the 2012 and 2013 cropping seasons at the Mitchell research farm of the University of Nebraska-Lincoln's Panhandle Research and Extension Center (PHREC) located in the North Platte River Valley, western Nebraska (41°56'N; 103°42'W). All sugar beet fields in which the research plots were established produced corn the preceding year. Sugar beet plots were subjected to reduced tillage (zone-tillage in particular) with a high percentage (> 30%) of corn residue remaining on the soil surface. During spring, and before performing the zone tillage operation (also done in spring), corn stalks were chopped by disking the fields. The zone tillage implement used for this study contained vertical shanks which cultivated soil to a depth of ca. 30.5 cm and a width of ca. 15-25 cm. With zone tillage, cultivation is concentrated to those zones which would constitute the new planting rows (Smith 2013). The zone tillage implement consisted of a single large coulter in front of each vertical shank (shanks positioned to attain 56 cm row spacing) that cuts corn residue. Positioned directly behind each vertical shank is a pair of wavy coulters that close the shank mark. Finally, behind the wavy coulters is a rolling basket that firms the soil surface to ensure improved seed-soil contact. All plots were treated with glyphosate twice early in the season for weed control.

The study was set up in a randomized complete block design with six replications. Treatment layout was a split plot arrangement with untreated seed and a seed-applied Poncho Beta (34.3% clothianidin and 4.6% beta-cyfluthrin) as the main plot experimental treatments and a high (86,487 plants/acre) and low plant density (61,776 plants/acre) as the split plot treatments.

These two planting densities represent a high and low end plant population for sugar beet production in the area (Yonts *et al.* 2013). Individual plots (main plot experimental units) contained eighteen rows of sugar beet with 56 cm row spacing, and measured 7 m x 10 m. Seed size was regular pellet (variety: Beta21RR25). Each plot was subsequently divided lengthwise in half so that each split plot experimental unit measured 7 m x 5 m and contained nine rows of sugar beets. The target plant density was attained by overplanting the plots to ca. 280,000 plants per hectare (within-row plant spacing of 6.35 cm). The plots were thinned twice to the desired stand during each cropping season in order to ensure the correct plant population. The first round of thinning was conducted on the 11-12<sup>th</sup> of June 2012, and 10-13<sup>th</sup> of June 2013. The second thinning was carried out on the 28-30<sup>th</sup> of June 2012, and 08-09<sup>th</sup> of July 2013.

**Beneficial arthropod activity density and ground beetle species richness.** Within each split plot, four pitfall traps were installed to monitor the activity density of beneficial resident epigeal arthropods. One pair of traps was oriented across the rows of each subplot, while the other was oriented lengthwise between the two center rows of each subplot. A sheet of metal flashing was installed between the two traps in each position, thereby linking pairs of traps. Flashings were installed to increase the rate of capture of beneficial arthropods. These metal flashings each measured ca. 165 cm x 30 cm, with ca. 15 cm buried below soil level. Pitfall traps were constructed by making a hole in the soil with a 107 mm diameter golf hole cutter and inserting a section of PVC piping (76 mm diameter and 150 mm high) into each hole to prevent soil from collapsing into the samples. A small disposable plastic cup (147 ml capacity), containing a mixture (ca. 38 ml) of ethylene glycol and water (1:3 ratio) as a killing and preservation agent was placed into each hole at the time of trap activation. A small amount of dishwashing liquid (ca. 10 ml) was added to this mixture to reduce surface tension. A tight-fitting plastic funnel (75 mm diameter on top and 25 mm diameter at the bottom) was placed on top of each cup to ensure capture of soil arthropods wandering into the traps. Each pitfall trap was subsequently covered

with a custom-manufactured plastic lid (250 mm diameter), leaving ca. 10 cm space between the lid and soil surface for arthropods to enter. The lids were affixed to a 40.7 cm x 8.9 cm piece of wood with 12.7 cm bolts attached to each end which were used to anchor the lid to the soil surface. Pitfall traps were left in the field for the duration of the growing season and capped with a tight-fitting lid when not activated.

Beneficial arthropod activity density was measured three times during the latter part of each growing season (samples removed on 03 July, 27 July and 29 August during 2012, and 05 July, 29 July and 30 August during 2013), with the traps left open for approximately seven days at a time. The taxa of beneficial epigeal arthropods sampled included selected beetle families (Carabidae, Staphylinidae, and Coccinellidae), spiders (Order: Araneae), harvestmen (Order: Opiliones), and centipedes (Class: Chilopoda). All of these are considered important predators of arthropod pests in agroecosystems (e.g. Weibull *et al.* 2003; Brewer and Elliot 2004; Eitzinger and Traugott 2011).

Due to the abundance and apparent diversity of ground beetles in the samples, coupled to their significance in agroecosystems worldwide (Holland and Luff 2000), they were identified to species. On account of significant differences in ground beetle activity density observed between the untreated and treated plots during 2013, three diversity indices (apart from measuring activity density) were calculated for this taxon: species richness, Simpson's diversity index, and Simpson's evenness. These diversity indices were calculated for each pitfall trap separately, where the number of beetles collected in each trap represents the cumulative total over the three collecting dates. A detailed description pertaining to the calculation of these indices is provided in Chapter 3.

**SBRA ratings and crop parameters.** To encourage the establishment of SBRA populations, plots were infested with SBRA adjacent to the two metal flashings within each plot. Aphid colonies were reared in a greenhouse on sugar beets grown in tall tree pots (Stuewe &



Sons, Inc.®). Each tree pot measured 10 cm wide by 36 cm high and had a 2.83 liter volume. A total of five sugar beet seeds were planted in each pot and subsequently thinned to two sugar beets per pot. Following this, five mature, apterous root aphids were introduced into each of three holes (16 cm diameter) made next to the sugar beet seedlings in each pot. Subsequently, the tree pots were incubated in a greenhouse at 23° C for three weeks (to allow buildup of SBRA populations), after which they were removed and field infestations conducted. All plots were inoculated with the soil from four pots at each flashing for a total of eight pots per subplot. Following the last arthropod sampling, four beets were removed next to the metal flashings and the level of SBRA infestation visually rated according to the 0-5 root rating scale developed by Hutchison and Campbell (1994). During both 2012 and 2013, root ratings were conducted on the 4<sup>th</sup> of September. All plots were machine harvested using a two-row sugar beet harvester. Only two rows out of nine in total from each subplot were harvested, which included rows four and five counted from the outside of each subplot inwards. A total of 7.62 meters was harvested from each of the two rows, from which two subsamples were collected ( $n = 8-10$  beets per subsample). For each subplot, sugar beet yield (tons/hectare), percentage sugar loss to molasses (SLM), sugar yield (kg/ha), and percentage sugar content were recorded. The two subsamples from each subplot were used to calculate both the percentage SLM and percentage sugar content at Western Sugar's tare laboratory (Scottsbluff), using standard industry procedures. Tonnage and kg sugar/ha was quantified for each subplot as a whole (i.e. one sample per subplot).

**Acceptance of SBRA as prey.** Because predation on SBRA colonies occurring on the secondary host (e.g. sugar beet) remains poorly understood, a controlled experiment was conducted to determine if the most dominant ground beetle species observed from this study accept this aphid species as prey. In 2013, six individual beetles (replicates) from ten commonly observed ground beetle species, observed during previous seasons (2010-2012: refer to Chapter 3), were captured live in sugar beet fields at the Mitchell research farm. Each individual was

starved for 24 hours, with only a moistened cotton wick provided as a source of moisture. Six field-collected apterous root aphids of varying ages were subsequently placed into a 20 ml glass scintillation vial with a single beetle. Vials were placed on their side to enable free movement of the beetles and prey, capped with a 70 mesh material that was affixed with a rubber band. These vials were used because previous experimentation (R.J. Pretorius: unpublished data) indicated that the SBRA is unable to scale the sides of the glass vials, which would have allowed them to escape predation. Vials containing the aphids and beetles were placed in a growth chamber at 23 °C (16 L: 8 D) for 24 hours, when the beetles were removed and the remaining aphids recorded.

**Statistical procedures.** The effect of plant density and seed-applied insecticides on beneficial arthropod activity density, root aphid populations, and crop parameters were evaluated by means of a two-way ANOVA implemented in SAS PROC GLIMMIX, version 9.2 (SAS 2008). This procedure was used to test for differences between the main plot factor (seed treatment) and the split plot factor (plant density), as well as any interactions that might exist between the two. For comparing beneficial arthropod activity density between the four treatments, the data from each pitfall trap were pooled (cumulative) over the three sampling dates of each year separately. Three sampling dates were chosen to collect a sufficient number of beneficial arthropods from each taxon for carrying out statistical analyses. This study did not aim at determining the seasonal activity and species composition of beneficial arthropods (an aspect which is addressed in Chapter 5), but rather aimed at evaluating the overall impact of plant density and insecticide-treated sugar beet seeds on these arthropods during the latter half of the season. Because the beneficial arthropod data represented direct counts, the data were fitted to either a Poisson or negative binomial distribution. Significantly different means among the treatments were separated using a Tukey ad hoc mean comparison test at the  $\alpha = 0.05$  level of significance. Mean comparisons with a marginal significance ( $P \leq 0.08$ ) are also discussed.

A one-way ANOVA was used to compare the three diversity indices for ground beetles between the treated and untreated plots, also with SAS PROC GLIMMIX, version 9.2 (SAS 2008). The same procedure was used to test for any differences in the number of SBRA's consumed between the ten most dominant ground beetle species. A Pearson's correlation was calculated in PROC CORR in SAS, version 9.2 (SAS 2008), to test the relationship between SBRA ratings and those crop yield parameters that showed a significant response to seed-applied insecticides. A similar procedure was used to determine the relationship between SBRA ratings and those ground beetle species that were significantly affected by seed-applied insecticides.

## Results

**Beneficial arthropod activity density and ground beetle species richness.** A total of 3,673 and 6,274 beneficial epigeal arthropods were collected in 2012 and 2013, respectively (Table 4.1). Very few lady beetles (total  $n = 7$ ) and harvestmen (total  $n = 15$ ) were collected during both seasons; therefore, these taxa were not considered for any further analyses. During 2012, the highest activity density was recorded for ground beetles, while centipedes were most abundant during the 2013. Centipede activity showed a marginal response to plant density during 2012, but not in 2013. Furthermore, they were not impacted by the seed-applied insecticide during either season (Figures 4.1 a and 4.1 b). During both seasons, seed-applied insecticide and plant density did not affect spider (Figures 4.1 c and 4.1 d) or rove beetle activity (Figures 4.1 e and 4.1 f). The same was true for ground beetle activity density during the 2012 cropping season (Figure 4.1 g). However, mean ( $\pm$  SEM) ground beetle activity during the 2013 cropping season (Figure 4.1 h), was significantly higher in the untreated plots ( $39.81 \pm 2.13$ ) compared to the insecticide-treated plots ( $31.11 \pm 2.82$ ). No interactions between the seed-applied insecticide and plant density were observed for any of the taxa.

Throughout this study, a total of 3,925 ground beetle specimens were collected in the pitfall traps, containing 36 species in 18 genera (Table 4.2). However, their numbers were slightly lower during the 2013 field season. Eight species made up ca. 90% of the total ground beetle abundance during 2012, while only five species constituted the same percentage during 2013 (Table 4.2). The most commonly collected species were *Harpalus erraticus* Say and *Bembidion quadrimaculatum oppositum* Say during the 2012 and 2013 seasons, respectively. During 2012, *H. erraticus* accounted for 30% of the total number of ground beetle specimens collected, but only 2% during the following year. In contrast, *B. quadrimaculatum oppositum* comprised 16% of the total number of ground beetles collected in 2012, but 58% of the total in 2013.

The number of ground beetle species caught between the seed-applied insecticide treatment and untreated plots were similar during both seasons (Table 4.3). In addition, there were no significant differences in Simpson's diversity index or Simpson's evenness between the treated and untreated plots during either year (Table 4.3). There was also little difference between the treated and untreated plots as far as the activity density of the most dominant ground beetle species was concerned (Table 4.4). However, one species, *B. quadrimaculatum oppositum*, had a significantly higher activity density ( $P = 0.01$ ) in the untreated plots ( $23.51 \pm 1.75$ ) compared to the treated plots ( $17.62 \pm 1.38$ ) during the 2013 season (Table 4.4), largely accounting for the observed difference in overall ground beetle activity density between these two treatments during this season. The dominance of this species also led to the low Simpson's diversity and evenness values calculated for this year. A correlation between the abundance of this ground beetle species and SBRA ratings during 2013 was non-significant ( $r = 0.23$ ,  $n = 48$ ,  $P = 0.12$ ).

**SBRA ratings and crop parameters.** During both years, the effect of seed treatment on root aphid populations was significant, while the effect of plant density was marginally significant only during 2013 (Figures 4.2 a and b). Sugar beet root aphid populations were significantly greater in untreated plots compared to the treated plots during both seasons (2012:

2.85  $\pm$  0.18 untreated vs. 2.31  $\pm$  0.18 treated plots; 2013: 2.93  $\pm$  0.19 untreated vs. 2.10  $\pm$  0.19 treated). No interaction between the seed-applied insecticide and plant density was observed for either year.

Seed treatment and plant density did not affect the percentage of sugar loss to molasses in either of the two seasons. In addition, no interaction was detected (Figures 4.2 c and d). Sugar content was not impacted by the treatment factors during 2012 (Figure 4.2 e); however, there was a marginal effect for seed treatment during 2013 (Figure 4.2 f) that resulted in a higher sugar content in insecticide-treated plots (11.30  $\pm$  0.24 untreated vs. 12.13  $\pm$  0.24 treated). There was a marginally significant negative correlation between sugar content and SBRA root ratings during this season ( $r = -0.36$ ,  $n = 24$ ,  $P = 0.08$ ), showing decreased sugar content with increased SBRA root ratings. Plant density affected tonnage with no interaction by insecticide (Figure 4.2 g). Higher root weights per plot were observed with lower plant density in 2012 (47.07  $\pm$  4.90 tons/ha low density vs. 36.30  $\pm$  4.90 tons/ha high density). However, neither plant density nor seed treatment affected tonnage during 2013, but there was a marginal interaction owing to slightly higher yield in the treated plots under the low plant density, but which was more similar to the untreated plots under the high plant density (Figure 4.2 h). In 2012, the effect of plant density on sugar yield (kg/ha) was significant, while sugar yield was unaffected by plant density and seed treatment during 2013 (Figures 4.2 i and j). As with tonnage, sugar yield was significantly higher in the lower plant density plots during 2012 (6,105  $\pm$  535 kg/ha low population vs. 4,704  $\pm$  535 kg/ha high population).

**Acceptance of SBRA as prey by selected ground beetle species.** All of the ten most-abundant ground beetle species tested readily accepted sugar beet root aphid as prey (Table 4.5), with no significant differences observed between the various species in their capacity to consume this aphid ( $F_{9,45} = 0.48$ ,  $P = 0.48$ ).

## Discussion and Conclusions

Contrary to the experimental hypothesis, insecticide seed treatments did not impact centipede, spider or rove beetle activity. Furthermore, the diversity, species richness, and evenness of ground beetles were not affected by the seed-applied insecticides. However, it did have an effect on one ground beetle species, *B. quadrimaculatum oppositum*, in 2013, but its impact was inconsistent between years owing to lower activity of this species during 2012. A reduction in prey numbers early in the season when insecticide seed treatments are reportedly most effective, and/or the indirect or direct toxicity through either imbibing prey exposed to the systemic insecticide or ingestion of treated vegetable matter, would explain a reduction in predatory numbers later in the season (Albajes *et al.* 2003). However, no such effect was observed in this study for centipedes, spiders and rove beetles, despite lower SBRA ratings in the treated plots. The results obtained are similar to those of Albajes *et al.* (2003) who did not observe differences in the abundance of spiders and ground beetles caught via pitfall trapping between imidacloprid treated and untreated plots under corn production; however, lower numbers of Staphylinidae were observed in their treated plots. These results are also in accordance with those of Krauter *et al.* (2001) who saw no impact of Gaucho® (imidacloprid) seed treatments on the late-season abundance of natural enemies sampled in sorghum. Seagraves and Lundgren (2011) also did not observe an effect of soybean seeds treated with thiamethoxam on spider abundance. However, other predatory taxa (Chrysopidae and Nabidae), as well as the overall predatory abundance, was reduced by the seed treatments in their study.

With this study, a complex of generalist natural enemies was sampled. While all individuals from the selected taxa were collected and enumerated, it is noteworthy that not all of the species in each taxon are strictly predatory. For example, rove beetles, certain ground beetle species, and even some spider species consume non-animal food (e.g. seeds, pollen and fungi). The fact that the sampled arthropods remained largely unaffected by the insecticide seed

treatments, suggests that either the omnivorous/phytophagous species did not supplement their diet with sugar beet vegetable matter to any significant degree, or, if this was the case, the impact of the systemic insecticide has diminished by the time the beneficial arthropods were first sampled (e.g. Westwood *et al.* 1998). This study was designed to measure beneficial arthropod activity density during mid-season when migrating SBRA colonize sugar beets and initiate colonies. Therefore, the early-season effects of seed treatments on beneficial arthropods were not assessed, and could have been significant. This is because the direct toxicity of the insecticide will be highest early in the season. The plots used in this study were also relatively small in their dimensions, and it is possible that recolonization by beneficial arthropods could happen rapidly following initial declines in their numbers. Finally, seed dressings are reportedly less toxic to natural enemies, in general, as opposed to insecticide foliar sprays which are more harmful (Croft 1990).

Ground beetles comprised a large component of the total number of epigeal beneficial arthropods collected during this study, especially during the first year. There is considerable interest in this group because of their contributions to pest and weed management in agroecosystems (Luff 2002). In their review on the impact of agriculture on ground beetle assemblages in temperate agroecosystems, Holland and Luff (2000) concluded that ground beetle assemblages within cropping systems are usually composed of ca. 30 species, of which usually less than 10 species dominate. This view was supported by our results in which 30 and 25 species were collected during 2012 and 2013, respectively. Furthermore, we found less than ten dominant species during both years. It was this dominance by only a few species each year that led to low Simpson's diversity and evenness values. Several of the most abundant ground beetle species collected in this study have been previously reported as abundant in agroecosystems in North America and elsewhere, highlighting their importance to agroecosystems. Examples include *B. quadrimaculatum* in alfalfa, carrots, corn, potatoes, soybeans, and wheat (Esau and Peters 1975;

Best and Beegle 1977; Hsin *et al.* 1979; Boivin and Hance 1994; Ellsbury *et al.* 1998; Kinnunen and Tiainen 1999; Melnychuk *et al.* 2003; Floate *et al.* 2007; Bourassa *et al.* 2008; Bourassa *et al.* 2010), *B. rapidum* (LeConte) in corn, soybeans, and wheat (Best and Beegle 1977; Hsin *et al.* 1979; Clark *et al.* 2006), *H. pensylvanicus* (DeGeer) in alfalfa, corn, millet, pasture grass, sorghum, soybeans, sunflowers, and wheat (Rivard 1966; Kirk 1971; Best and Beegle 1977; Hsin *et al.* 1979; Weiss *et al.* 1990; Tonhasca 1993; Pavuk *et al.* 1997; Ellsbury *et al.* 1998; Clark *et al.* 2006; Miller and Peairs 2008), *Stenolophus comma* (Fabricius) in alfalfa, beans, corn, potatoes, sainfoin, and wheat (Hsin *et al.* 1979; Lester and Morrill 1989; Bourassa *et al.* 2008), *E. anceps* in corn, soybeans, and wheat (Clark *et al.* 2006), *H. erraticus* in corn (Kirk 1971), *Amara carinata* (LeConte) in beans, corn, and potatoes (Kirk 1971; Floate *et al.* 2007; Bourassa *et al.* 2008), *A. farcta* LeConte in alfalfa, beans, corn, potatoes, sainfoin, and wheat (Lester and Morrill 1989; Bourassa *et al.* 2008; Bourassa *et al.* 2010), *B. tetracolum* Say in cabbage (Armstrong and McKinlay 1997; Prasad and Snyder 2004), and *H. amputatus* Say in alfalfa, corn, millet, sainfoin, sorghum, sunflower, and wheat (Lester and Morrill 1989; Miller and Peairs 2008).

With the exception of one species (*B. quadrimaculatum oppositum*) in 2013, the activity of the remaining ground beetle species was not influenced by seed treatments. Pavuk *et al.* (1997), who studied ground beetle communities in vegetationally diverse corn agroecosystems, also saw very little difference in species-specific activity density between different treatments. Furthermore, the fact that the majority of the ground beetle species collected in this study (as well as the remaining beneficial taxa) did not exhibit higher numbers in the untreated plots where SBRA abundance was higher is not surprising, considering the fact that generalist natural enemies rarely respond numerically to any single prey species (Symondson *et al.* 2002a).

The high rate of capture of *B. quadrimaculatum oppositum* during 2013 accounted for both the overall difference in ground beetle activity between the treated and untreated plots, as well as for the lower biodiversity indices (both treatments) relative to 2012. This demonstrates



that beneficial arthropod activity density and species assemblage can vary greatly between localities and seasons (e.g. Albajes *et al.* 2003; Bourassa *et al.* 2008). It also highlights the importance of evaluating key taxa on the species (or generic) level, rather than on an ordinal or family level. *Bembidion quadrimaculatum oppositum* is a well-documented predator of arthropods (e.g. Best and Beegle 1977; Grafius and Warner 1989; Baines *et al.* 1990); therefore, it is reasonable to hypothesize that it responded to the elevated SBRA numbers in the untreated plots. However, the results from a correlation between this species and SBRA ratings did not support this, suggesting that it might have responded to a different suite of arthropod prey that was affected by the seed treatments in the treated plots.

With the exception of centipedes in 2012, we found no effect of plant density on the activity density of epigeal beneficial arthropods. The observations from this study agree with those made by Boiteau (1984) who observed no difference in the abundance of ground beetles, spiders, and rove beetles between 15- and 36-cm within-row seed spacings in potatoes. Mayse (1978) did observe higher natural enemy abundance between low density and high density soybeans, but they manipulated between-row spacing, which led to changing microclimatic conditions between these two treatments due to differences in the degree of soil coverage by foliage (i.e. more open spaces with increased row spacing). In addition, they sampled a different natural enemy complex, mainly confined to the above-soil parts of the crop. All plots in this study were subjected to the same agricultural practices and both plant densities tested in this study are common for the area of research (Yonts *et al.* 2013). Nonetheless, due to the leafy structure of the crop and the fact that between-row spacing was not altered, sugar beet plants in the low population plots were still likely able to compensate and produce enough leaf biomass to cover the soil surface as it would in the high plant density plots, thus leading to minimal microclimatic differences. In fact, there was no visual distinction between the low and high population treatments.

Similar to the findings of Strausbaugh *et al.* (2010), insecticide seed treatments significantly reduced SBRA populations during both seasons in this study. However, infestation levels remained moderately high ( $> 2$  on the root rating scale) in the treated plots. These levels of SBRA aphid infestation would still contribute to yield loss (Hutchison and Campbell 1994), and, therefore, our results support the statement made by Dewar and Cooke (2006) that seed treatments will be less effective against late-season pests such as the SBRA. Alternatively, it is possible that the mortality of root aphids by seed treatments was additive with the action of natural enemies preying on immigrants earlier in the season when colonization of sugar beet took place, thereby resulting in greater suppression of aphid populations in treated relative to untreated plots. Higher sugar content was measured in the treated plots only during 2013. A correlation between SBRA root ratings and this parameter indicated a moderately strong negative relationship, indicating that SBRA, at least in part, contributed to the decreased sugar content. Lower sugar content is expected as a consequence of higher SBRA pressure (Hutchison and Campbell 1994). However, the results from this study are too inconsistent to draw any definitive conclusions on the positive impact of seed-applied insecticides on this yield parameter, owing to similar sugar content between the treated and untreated plots in 2012 (despite significantly higher SBRA populations in the insecticide-treated plots). For the remaining yield parameters, SBRA appeared to have little impact as illustrated by a lack of significant differences between the treated and untreated plots, despite higher aphid pressure in the untreated plots. Differences in root yield and sugar yield between the low and high plant densities in 2012, were opposite to what was expected (i.e. higher yields under higher beet populations). During this season, the general area of the field in which the research plots for this study was established showed signs of water stress in the sugar beet crop. It is possible that increased drainage (or some other unknown factor) resulted in higher competition for moisture between individual plants, which would have put plants in the lower plant population at an advantage.

No-choice prey experiments have been criticized as being unrealistic, because potential predators are starved ahead of time and are, therefore, more likely to accept prey they would normally not prefer under natural conditions. Nonetheless, the high rate of predation observed for all ground beetle species tested (with no differences in their consumption), indicate that these generalists predators are capable of feeding on SBRA. This warrants further investigation into the contribution of these predators to SBRA management.

Seed-applied neonicotinoids appear to significantly reduce pests with a minimal impact on edaphic beneficial arthropods in sugar beets. The only exception to this was observed with the ground beetle species, *B. quadrimaculatum oppositum*, which was significantly suppressed by seed-applied insecticides during one year of the study. For the SBRA, however, the level of suppression cannot be considered optimal for SBRA management in practice. With minor exceptions, plant density also had little impact on these organisms.

**Table 4.1:** Total number of beneficial arthropods (by taxon) collected by means of pitfall trapping during 2012 and 2013.

Beneficial arthropod taxon	Total number collected <sup>a</sup>	
	2012	2013
Araneae (spiders)	411	558
Carabidae (ground beetles)	2,205	1,720
Chilopoda (centipedes)	574	2,607
Coccinellidae (lady beetles)	7	0
Opiliones (harvestmen)	3	12
Staphylinidae (rove beetles)	473	1,377
Total	3,673	6,274

<sup>a</sup> Total number of individuals collected from  $n = 288$  pitfalls.

**Table 4.2:** Percentage abundance of ground beetle (Coleoptera: Carabidae) species collected during the 2012 and 2013 field seasons.

Species	% Total	
	2012 <sup>a</sup>	2013 <sup>b</sup>
<i>Acupalpus partiaris</i> (Say)	-	0.06
<i>Agonum placidum</i> (Say)	0.27	0.58
<i>Amara carinata</i> (LeConte)	9.98*	1.86
<i>Amara farcta</i> LeConte	11.61*	-
<i>Amara quenseli quenseli</i> (Schönherr)	-	0.06
<i>Anisodactylus carbonarius</i> (Say)	0.05	-
<i>Bembidion nitidum</i> (Kirby)	0.32	0.41
<i>Bembidion obscurellum obscurellum</i> (Motschulsky)	-	0.06
<i>Bembidion quadrimaculatum oppositum</i> Say	16.01*	57.79*
<i>Bembidion rapidum</i> (LeConte)	1.50	4.30*
<i>Bembidion tetracolum tetracolum</i> Say	2.54	16.16*
<i>Brachycellus congener</i> (LeConte)	0.05	-
<i>Brachycellus rupestris</i> (Say)	0.05	-
<i>Chlaenius tricolor tricolor</i> Dejean	0.05	1.98
<i>Cicindela punctulata punctulata</i> Olivier	1.90	0.35
<i>Cratacanthus dubius</i> (Palisot de Beauvois)	-	0.06
<i>Dicheirotichus cognatus</i> (Gyllenhal)	0.05	-
<i>Dyschirius globulosus</i> (Say)	-	0.06
<i>Elaphropus anceps</i> (LeConte)	5.26*	8.66*
<i>Harpalus amputatus amputatus</i> Say	2.68*	0.23
<i>Harpalus caliginosus</i> (Fabricius)	0.23	0.17
<i>Harpalus erraticus</i> Say	29.89*	1.63
<i>Harpalus herbivagus</i> Say	0.18	0.17
<i>Harpalus pensylvanicus</i> (DeGeer)	5.40*	2.15*
<i>Harpalus reversus</i> Casey	0.59	0.23
<i>Harpalus somnulentus</i> Dejean	0.09	-
<i>Lebia bivittata</i> (Fabricius)	0.05	-
<i>Microlestes linearis</i> (LeConte)	0.45	1.63
<i>Poecilus chalcites</i> (Say)	0.05	-
<i>Poecilus lucublandus</i> (Say)	1.22	0.23
<i>Poecilus scitulus</i> LeConte	0.45	-
<i>Pterostichus femoralis</i> (Kirby)	0.05	-
<i>Pterostichus melanarius melanarius</i> (Illiger)	-	0.06
<i>Pterostichus permundus</i> (Say)	0.27	0.06
<i>Stenolophus comma</i> (Fabricius)	8.75*	1.05
<i>Stenolophus lineola</i> (Fabricius)	0.05	-

Sum	2,205	1,720
Number of species	30	25

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<sup>a</sup> A total of 2,205 ground beetles collected during three sampling dates.

<sup>b</sup> A total of 1,720 ground beetles collected during three sampling dates.

\* Ground beetle species making up ca. 90% of the total captures within a specified year.

**Table 4.3:** Comparisons of ground beetle (Coleoptera: Carabidae) species richness, Simpson's diversity index, and Simpson's evenness between seed-applied insecticide sugar beet and untreated sugar beet during 2012 and 2013.

	Species richness ( <i>S</i> )		Simpson's diversity ( <i>D</i> )		Simpson's evenness ( <i>E</i> )	
	2012	2013	2012	2013	2012	2013
Untreated	10.46 ± 0.38	7.17 ± 0.32	5.62 ± 0.23	2.55 ± 0.17	0.54 ± 0.02	0.36 ± 0.02
Treated	10.13 ± 0.38	7.04 ± 0.32	4.99 ± 0.23	2.74 ± 0.17	0.50 ± 0.02	0.40 ± 0.02
df	1, 5	1, 5	1, 5	1, 5	1, 5	1, 5
<i>F</i>	0.38	0.09	3.73	0.99	2.54	2.35
<i>P</i>	0.56	0.78	0.11	0.37	0.17	0.19

**Table 4.4:** Mean ( $\pm$  SEM) activity density of the most abundant ground beetle (Coleoptera: Carabidae) species collected by means of pitfall trapping in seed-applied insecticide sugar beet and untreated sugar beet in western Nebraska.

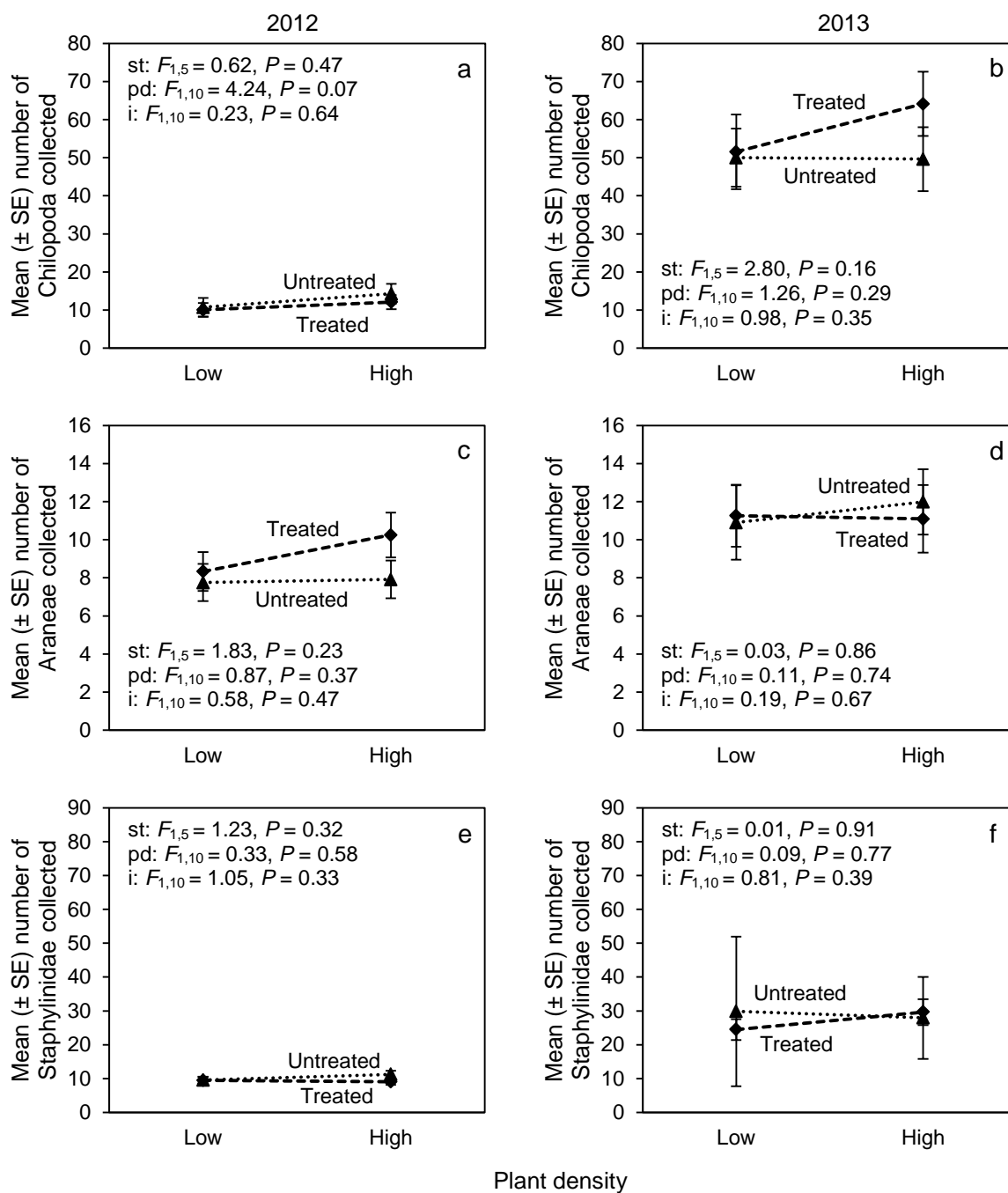
	Treatment		df	<i>F</i>	<i>P</i>
	Untreated	Treated			
2012					
<i>Amara carinata</i> (LeConte)	4.58 ± 0.58	4.52 ± 0.57	1, 5	0.01	0.94
<i>Amara farcta</i> LeConte	3.77 ± 0.89	5.96 ± 1.35	1, 5	3.42	0.12
<i>Bembidion quadrimaculatum oppositum</i> Say	7.13 ± 0.88	7.37 ± 0.90	1, 5	0.04	0.86
<i>Elaphropus anceps</i> (LeConte)	2.50 ± 0.85	1.50 ± 0.53	1, 5	3.34	0.13
<i>Harpalus amputatus amputatus</i> Say	0.95 ± 0.31	1.25 ± 0.39	1, 5	0.37	0.57
<i>Harpalus erraticus</i> Say	11.36 ± 2.51	13.52 ± 2.97	1, 5	0.37	0.57
<i>Harpalus pensylvanicus</i> (DeGeer)	2.63 ± 0.44	2.26 ± 0.40	1, 5	0.38	0.56
<i>Stenolophus comma</i> (Fabricius)	1.95 ± 0.92	3.25 ± 1.50	1, 5	0.61	0.47
Other	5.08 ± 0.60	4.42 ± 0.54	1, 5	1.11	0.34
2013					
<i>Bembidion quadrimaculatum oppositum</i> Say	23.51 ± 1.75	17.62 ± 1.38	1, 5	13.92	0.01
<i>Bembidion rapidum</i> (LeConte)	1.26 ± 0.44	1.36 ± 0.47	1, 5	0.03	0.86
<i>Bembidion tetracolum tetracolum</i> Say	6.36 ± 1.17	4.69 ± 0.89	1, 5	2.69	0.16
<i>Elaphropus anceps</i> (LeConte)	3.48 ± 0.63	2.51 ± 0.48	1, 5	1.91	0.23
<i>Harpalus pensylvanicus</i> (DeGeer)	1.04 ± 0.21	0.50 ± 0.15	1, 5	4.37	0.09
Other	3.69 ± 0.46	4.10 ± 0.49	1, 5	0.53	0.50

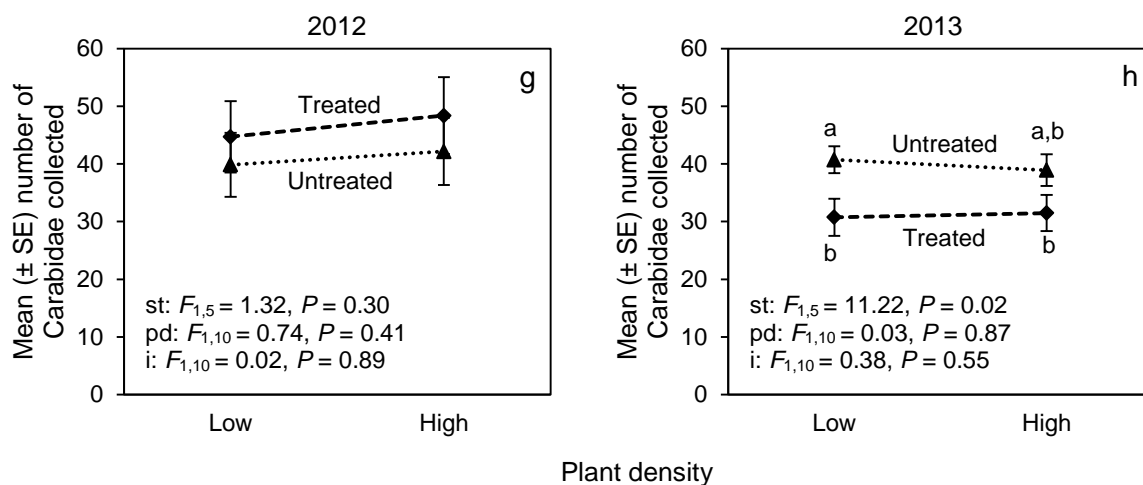


**Table 4.5:** Mean ( $\pm$  SEM) sugar beet root aphids accepted as prey by ten abundant ground beetle (Coleoptera: Carabidae) species in western Nebraska under controlled conditions.

Ground beetle species	Aphid consumption <sup>a</sup>
<i>Cicindela punctulata punctulata</i> Olivier	6.00 $\pm$ 0.00
<i>Bembidion obscurellum obscurellum</i> (Motschulsky)	6.00 $\pm$ 0.00
<i>Harpalus erraticus</i> Say	6.00 $\pm$ 0.00
<i>Harpalus pensylvanicus</i> (De Geer)	5.83 $\pm$ 0.17
<i>Harpalus amputatus amputatus</i> Say	5.83 $\pm$ 0.17
<i>Bembidion tetracolum tetracolum</i> Say	5.83 $\pm$ 0.17
<i>Bembidion quadrimaculatum oppositum</i> Say	5.50 $\pm$ 0.50
<i>Bembidion nitidum</i> (Kirby)	4.83 $\pm$ 0.65
<i>Bembidion rapidum</i> (LeConte)	4.67 $\pm$ 0.49
<i>Elaphropus anceps</i> (LeConte)	4.17 $\pm$ 0.65

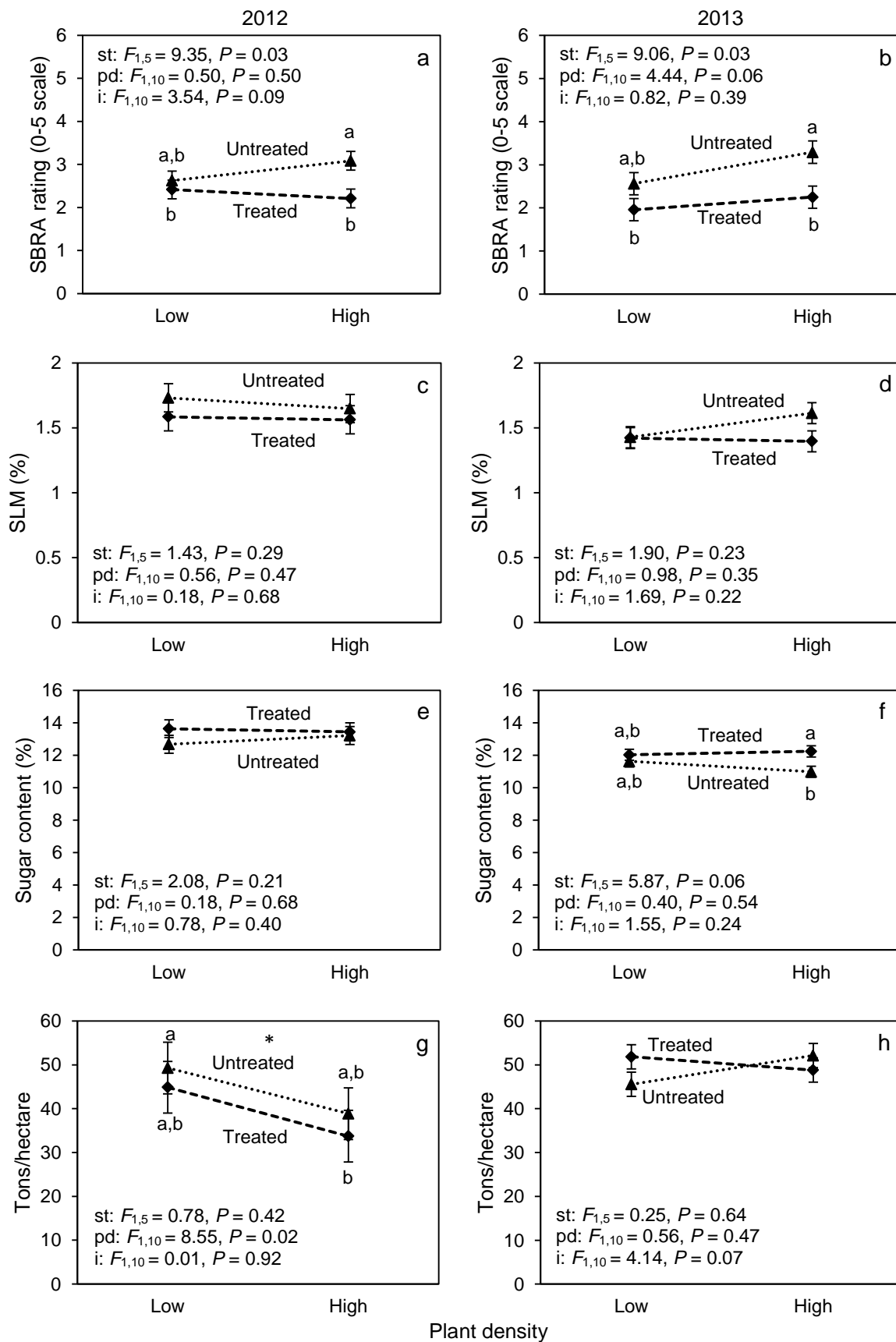
<sup>a</sup>  $n = 6$  root aphids presented to each ground beetle ( $n = 6$  replicates for each ground beetle species).

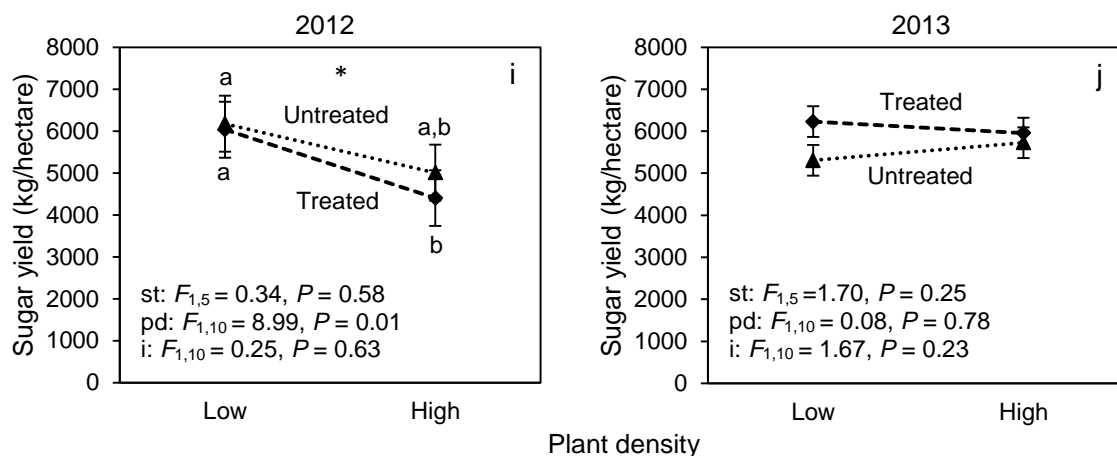




‘st’ = Seed treatment, ‘pd’ = plant density, ‘i’ = seed treatment x plant density interaction.

**Figure 4.1:** Effect of seed-applied insecticide (Poncho Beta) and plant density of sugar beet on centipede (a and b), spider (c and d), rove beetle (e and f), and ground beetle activity density (g and h), as revealed by a two-way ANOVA. Treatments were: i) untreated seed planted at a low plant density (25,000 plants/acre), ii) untreated seed planted at a high plant density (45,000 plants/acre), iii) treated seed planted at a low plant density, iv) treated seed planted at a high plant density. Dotted lines with triangles represent untreated seed treatments, while dashed lines with diamonds represent seed-applied insecticide treatments. Data for 2012 are contained in the left-hand figures (a, c, e, and g), while data for 2013 is contained in the right-hand figures (b, d, f, and h). Means ( $\pm$  SEM) with different lower-case letters are significantly different at  $\alpha = 0.05$  (post-hoc Tukey mean comparison test).





‘st’ = Seed treatment, ‘pd’ = plant density, ‘i’ = seed treatment x plant density interaction. \* = Significant differences between the low and high plant densities.

**Figure 4.2:** Effect of seed-applied insecticide (Poncho Beta) and plant density of sugar beet on sugar beet root aphid populations (a and b), sugar loss to molasses (c and d), sugar content (e and f), tonnage (g and h), and sugar yield (i and j) as revealed by a two-way ANOVA. Treatments were: i) untreated seed planted at a low plant density (25,000 plants/acre), ii) untreated seed planted at a high plant density (35,000 plants/acre), iii) treated seed planted at a low plant density, iv) treated seed planted at a high plant density. Dotted lines with triangles represent untreated seed treatments, while dashed lines with diamonds represent seed-applied insecticide treatments. Data for 2012 are contained in the left-hand figures (a, c, e, g, and i), while data for 2013 is contained in the right-hand figures (b, d, f, h, and j). Means ( $\pm$  SEM) with different lower-case letters are significantly different at  $\alpha = 0.05$  (post-hoc Tukey mean comparison test).

## CHAPTER 5

### THE IMPACT OF TILLAGE ON BENEFICIAL EPIGEAL ARTHROPOD COMMUNITIES AND THEIR ASSOCIATED ECOSYSTEM SERVICES IN SUGAR BEETS

#### Introduction

Much research has focused on the role of beneficial arthropods (i.e. predators, parasitoids, and herbivores) in agroecosystems. There is consensus that these organisms should be conserved and enhanced to benefit from the ecosystem services they provide (Pimentel *et al.* 1992; Way and Heong 1994; Pickett and Bugg 1998; Altieri 1999; Cromar *et al.* 1999; Kendall 2003; Hooper *et al.* 2005; Zehnder *et al.* 2006; Fiedler *et al.* 2008; Holloway *et al.* 2008; Isaacs *et al.* 2008; Godfray *et al.* 2010; Benayas and Bullock 2012; Woodcock *et al.* 2014). The term ‘ecosystem services’ was defined by Daily (1997) as: “the conditions and processes through which natural ecosystems, and the species that make them up, sustain and fulfill human life”. There are a multitude of ecosystem services, but arthropod-mediated ecosystem services (AMES) include the biological control of arthropod pests (predation and parasitism) and weeds (Isaacs *et al.* 2008), as well as pollination of several crops. In this way, AMES contributes to decreased pesticide input, and increased crop sustainability and yield (Kendall 2003; Griffiths *et al.* 2008; Isaacs *et al.* 2008; Woodcock *et al.* 2014). Highlighting the importance of AMES, Losey and Vaughn (2006) estimated its value at almost \$8 billion annually in the United States; \$4.5 billion of which is due to the biological control of insect pests.

Several beneficial soil-dwelling arthropod taxa have been recorded from arable land, including spiders (Araneae), centipedes (Chilopoda), beetles (Coleoptera), harvestmen (Opiliones), earwigs (Dermaptera), true bugs (Heteroptera), neuropterans (Neuroptera), flies (Diptera), and ants (Hymenoptera). Many of these are polyphagous and have the capability to

feed on a range of pest species (Kendall 2003). Farming operations, such as tillage, can have a profound impact on beneficial arthropod community structure and abundance (Stinner and House 1990; Weiss *et al.* 1990; Cárcamo, *et al.* 1995; Heimbach and Garbe 1996; Clark *et al.* 1997; Altieri 1999; Andersen 1999; Holland and Luff 2000; Holland and Reynolds 2003; Kendall 2003), and by implication, their associated degree of ecosystem services. As such, the impact of farming practices on these organisms should be taken into account. Soil tillage can impact beneficial arthropod survival either through direct mortality or by modifying prey availability or the physical environment (e.g., when crop residue is left on the soil surface) (Holland and Luff 2000; Kendall 2003; Holland 2004; Thorbek and Bilde 2004).

With the introduction of glyphosate-tolerant sugar beet varieties in 2008, the use of zone tillage in sugar beet has increased. With this tillage system, residue from the previous crop is moved away from the row and a narrow zone (ca. 15-25 cm), where the new crop rows will be planted, is cultivated (Smith 2013). This form of reduced/conversion tillage is less intensive compared to conventional tillage systems that involve moldboard plowing where soil inversion takes place along with nearly complete burial of residue (Dickey *et al.* 1992). Additionally, zone tillage lowers operational and labor costs (Smith *et al.* 1995), and protects against wind and water erosion. Zone tillage is practiced widely for sugar beet production in Nebraska, Colorado, and southern Wyoming, with 60% of the Nebraska sugar beet crop produced by this method (Smith 2013). This is due to the ease of controlling weeds with a single active ingredient (glyphosate), without the need for additional cultivation practices (Smith 2013) or the use of herbicide tank mixtures.

Unfortunately, glyphosate resistant weed populations recently have been documented in many sugar beet production regions of the United States (Shaw *et al.* 2011), necessitating an integrated approach to weed management in which beneficial arthropods could play an important role. Furthermore, an increased emphasis on improving sustainability of production systems

places more emphasis on natural pest control as opposed to high-input agriculture that relies heavily on agrochemicals (Holland and Luff 2000).

Post-dispersal weed-seed feeding (i.e. feeding on seeds shed from the parent plant) by vertebrates and invertebrates is widely recognized as an important contributing factor for weed management in agroecosystems (House and Brust 1989; Reader 1991; Cardina *et al.* 1996; Cromar *et al.* 1999; Kromp 1999; Menalled *et al.* 2000; Tooley and Brust 2002; Harrison *et al.* 2003; Honek *et al.* 2003; Westerman *et al.* 2003; Honek *et al.* 2005; Heggenstaller *et al.* 2006; Chauhan *et al.* 2010). By consuming weed seeds, the number of seeds surviving and germinating in the seed bank is reduced (Brust and House 1988; Crawley 1992; Cromar *et al.* 1999; Crawley 2000; Tooley and Brust 2002; Honek *et al.* 2003; Gallant *et al.* 2005; Landis *et al.* 2005; Bohan *et al.* 2011). This can change weed community composition (Crawley 2000; Tooley and Brust 2002). Not only do weeds compete with the crop for nutrients, moisture, and sunlight, but they also can act as a secondary host to certain insect pest species (Hein and Johnson 2001; Capinera 2005).

Apart from impacting beneficial arthropod species assemblages and abundance directly, the ecosystem services such as weed seed consumption and invertebrate predation also can be strongly influenced by the tillage practice. As an example, Brust and House (1988) reported that the rate of weed seed removal by invertebrates (ground beetles, crickets, and ants) and rodents was twice as high in no-till soybeans compared to moldboard plowed fields. Cromar *et al.* (1999) also measured higher weed seed consumption in no-tillage and moldboard plowed fields (averaging 32% weed seed consumption) as opposed to chisel-plowed fields (averaging 24% weed seed consumption). The observed differences in the degree of ecosystem services rendered between cultivation regimes might be a direct consequence of differing beneficial arthropod abundance brought about by the direct and indirect effects of tillage on their populations. It is also likely that the effects of tillage will have an impact on other ecosystem functions rendered by



beneficial arthropods, such as predation. However, the impact of tillage on other types of AMES, particularly on predation rates of prey, has received less attention.

It is imperative to assess the impact of farming operations, such as tillage, on ecosystem services and the organisms providing them, for the goal of identifying and developing better techniques to conserve and enhance these services (Altieri 1999). This is especially important considering the concerns over the long-term sustainability of our ecologically simplified agroecosystems (Altieri 1999). Several studies have investigated differences between species richness, abundance, and distribution of beneficial arthropods in various agroecosystems; however, few have investigated the degree of ecosystem function across management regimes (Griffiths *et al.* 2008).

We hypothesize that reduced tillage will improve the ecosystem function of resident beneficial arthropods in sugar beet agroecosystems in western Nebraska. Residue cover left on the soil surface in zone tillage systems should favor beneficial arthropods, leading to increased prey and post-dispersal weed seed removal rates as a result of their higher abundance. Our target taxon for inquiry was ground beetles as they are reportedly sensitive to cultivation (Holland 2004); therefore, they would serve as bio-indicators for monitoring the impact of cultivation on beneficial arthropods (Kromp 1999; Holland and Luff 2000). Furthermore, these insects are considered efficient generalist predators of both arthropod pests and weeds.

## **Materials and Methods**

**Study site:** The study was conducted during the 2012 and 2013 field seasons at the University of Nebraska-Lincoln's Mitchell research farm, which forms part of the Panhandle Research and Extension Center (PHREC) located in the North Platte River Valley, western Nebraska (41°56'N; 103°42'W). Experiments were carried out in sugar beet plots established as part of a multi-year study investigating the long-term impact of crop rotations that include corn,

dry beans and sugar beet. This study was initiated in 2007, and included zone tillage, conventional tillage, and no-tillage conditions. The plots were established in a sugar beet field (cv. 'Beta21RR25') following corn cultivation. Each tillage practice was replicated five times in a randomized complete block design, with individual plots measuring 12 rows (6.7 m) by 65.2 m.

Before any of the aforementioned cultivation operations (conventional and zone tillage) took place, corn stalks were chopped by disking the fields. Following this, a zone tillage implement was used to establish the planting rows in the zone tillage plots. This implement consists of a single large coulter (to cut surface corn residue), positioned in front of each vertical shank (30.5 cm depth). Positioned directly behind each shank is a pair of wavy coulters which function to close the shank marks. Behind the wavy coulters are rolling baskets which compress and firm the seedbed to ensure good seed-soil contact. In contrast, the conventional tillage operation consisted of the use of a moldboard plow (ca. depth of 30.5 cm). Therefore, most of the corn residue was buried below soil level in the conventional tilled plots. All research plots were treated twice with glyphosate early in the season for weed control.

**Beneficial arthropod activity density:** Beneficial epigeal arthropods were sampled throughout the field season (May-September) by means of pitfall trapping in the conventional- and zone tillage sugar beet plots ( $n = 6$  traps/plot). During both years, the percentage of surface residue was estimated at two separate locations within each plot for both tillage systems using the line-transect method (Shelton and Jasa 2009). This action was performed on 4 August 2012 and 18 July 2013. To increase the capture efficiency of pitfall traps, pairs of traps were linked by means of a metal flashing buried into the soil and running perpendicular to the sugar beet rows. The metal flashings measured ca. 165 cm x 30 cm, with ca. 15 cm buried below soil level. They were installed in such a way that the edges of the flashings nearly touched the perimeter of the pitfall traps. Pitfall traps were constructed by making a hole in the soil with a 107 mm diameter golf hole cutter and inserting a section of PVC pipe (76 mm diameter and 150 mm high) into each

hole to prevent soil from collapsing. A small disposable plastic cup (147 ml capacity), containing a mixture (approximately 38 ml) of ethylene glycol and water (1:3 ratio) as a killing and preservation agent, was placed into each hole at the time of trap activation. A small amount of dishwashing liquid (ca. 10 ml) was also added to this mixture to reduce surface tension. A tight-fitting plastic funnel (75 mm at the top and 25 mm at the bottom) was placed inside the PVC pipe and on top of each cup to ensure capture of soil arthropods. Each pitfall trap was subsequently covered with a plastic lid (250 mm diameter), leaving ca. 10 cm between the lid and soil surface for arthropods to enter. The plastic lids were secured to a 406.5 mm x 89 mm piece of wood with 127 mm bolts attached to each end which were used to anchor the lid to the soil surface. Pitfall traps were left in the field for the duration of the growing season and capped with a tight-fitting lid when not activated.

The pitfall traps were activated six times throughout both the 2012 (samples removed on 24 May, 08 June, 05 and 29 July, 14 August, and 11 September) and 2013 (samples removed on 30 May, 19 June, 10 and 30 July, 21 August, and 12 September) growing seasons. The traps were left open for five days during each activation period. All samples were collected and stored in a cooler at 4-5 °C until they could be processed. Although the emphasis was on ground beetles, several other taxa of beneficial epigeal arthropods were also sampled, including two additional beetle families (Staphylinidae and Coccinellidae), spiders (Order: Araneae), harvestmen (Order: Opiliones), and centipedes (Class: Chilopoda). These taxa were chosen based on their importance in agroecosystems as natural enemies of arthropod pests and weeds, and because they were the most commonly observed beneficial taxa (with the exception of Coccinellidae) in previous studies (see Chapters 3 and 4).

The seasonal abundance of four beneficial arthropod taxa (Carabidae, Chilopoda, Araneae, and Staphylinidae) were compared over the season ( $n = 6$  sample dates) and between the two tillage practices by using a two-way ANOVA with repeated measures implemented in SAS

PROC GLIMMIX, version 9.2, SAS (2008). The data from pairs of traps were combined ( $n = 3$  traps/plot) due to low captures during certain sampling periods. For Chilopoda, the first sampling dates in both years were excluded from the analyses because few individuals were sampled during these periods. The data for the different taxa were fitted to one of two distributions (negative binomial or Poisson distribution) depending on the goodness of fit. Various covariance structures were applied to the data, and the appropriate structure (for each taxon within a particular year) was chosen based on the lowest AICc value. Significant differences between the means for both sample date and tillage effects were separated with a protected ad hoc LSD test ( $\alpha = 0.05$ ). Marginal significant differences ( $P \leq 0.08$ ) are also discussed.

**Ground beetle species richness and diversity:** Because of their higher activity density, ground beetles were identified to species level to compare species richness and diversity between treatments. Three diversity indices (apart from measuring activity density) were calculated; species richness ( $S$ ), Simpson's diversity index ( $D$ ), and Simpson's evenness ( $E$ ). A detailed description pertaining to the calculation of these indices is provided in Chapter 3. These diversity indices were calculated for each individual pitfall trap and for each sampling date separately. The data were compared between treatments by a two-way ANOVA with repeated measure analysis, similar to that described for comparing activity density above. The data were fitted to either to either a negative binomial or Poisson distribution, depending on the goodness of fit. Significantly different means for sampling time and tillage effects were separated with an ad hoc LSD pair-wise comparison test ( $\alpha = 0.05$ ).

**Post-dispersal weed seed removal from the field:** Weed seed removal rate by beneficial arthropods in sugar beet were compared by means of a split-plot experiment. The main plot treatments were tillage (two levels: conventional and zone tillage) and the split plot treatments were four different weed species. Weed species used included two grasses, yellow foxtail (*Setaria glauca* (L.)) and barnyardgrass (*Echinochloa crus-galli* (L.)), and two broad-leaf weeds, kochia

(*Kochia scoparia* (L.)) and common lambsquarters (*Chenopodium album* L.). These species are all common weeds in sugar beet production systems in the Central High Plains (May and Wilson 2006), and they have the capacity to reduce sugar beet yields. Furthermore, both lambsquarters and kochia act as secondary hosts for the sugar beet root aphid, *Pemphigus betae* Doane, an important economical pest of sugar beet. Additionally, herbicide resistance has been observed in barnyardgrass (Carey *et al.* 1997; Talbert and Burgos 2007; Juliano *et al.* 2010), kochia (Guttieri *et al.* 1995; Foes *et al.* 1999; Cranston *et al.* 2001; Crespo *et al.* 2014), and common lambsquarters (Darmency and Gasquez 1990; Parks *et al.* 1996; Westhoven *et al.* 2008).

Seeds from each weed species were fixed to the bottom of modified petri dishes (100 x 15 mm) using double-sided sticky tape (Scotch® 3M removable double sided tape). Each petri dish contained seeds of a single weed species, with twenty seeds/dish for the two grasses and thirty seeds/dish for the two broad-leaf weeds. Following attachment of the weed seeds to the sticky tape, fine gravel was added to coat the remaining sticky surface to prevent arthropods from becoming trapped. The seed dishes were placed into specially constructed vertebrate exclusion cages, designed to keep out potential non-invertebrate seed feeders. These cages measured ca. 61 cm x 15 cm x 13 cm (length x width x height), and were constructed of galvanized wire screen (11 x 11 mm screen size). The sides of the cages were buried to ca. 4 cm below soil level. Four seed dishes, each containing the seeds of a different weed species, were randomly placed into each exclusion cage. The seed dishes were buried into the soil so the outside rims were flush with the soil surface to allow easy access. Three exclusion cages were randomly placed between the two center rows of each plot. To control for other factors that could contribute to seed removal (i.e. environmental factors such as rain, wind, etc.), a control cage was included in each plot. Control cages were of the same construction as experimental cages; however, they were completely covered with a fine, Lumite mesh screen material, preventing access to all potential seed feeders from outside the cages. This experiment was repeated on three separate dates during both the

2012 (02-13 July, 06-15 August, and 06-15 September) and 2013 (02-12 July, 14-23 August, and 06-16 September) growing seasons. The seed dishes were left in the field for approximately ten consecutive days and then the number of damaged and missing seeds enumerated.

Because of low seed consumption rates during the first and last sampling dates of both years, data for each exclusion cage was pooled across the three sampling dates to arrive at the total number of seeds removed per cage for each weed species in that year. Data for the two years were fitted to a beta distribution (to attain the proportion of seeds consumed) and analyzed separately by means of a two-way ANOVA using the PROC GLIMMIX procedure in SAS version 9.2 (SAS 2008). This procedure was used to test for significant tillage and weed species differences and any interactions. Significantly different means among the two factors were separated using a protected LSD ad hoc test at the  $\alpha = 0.05$  level of significance.

**Weed seed choice feeding assays:** During the 2013 growing season, four ground beetle species, *Harpalus pensylvanicus* (De Geer), *H. erraticus* Say, *H. amputatus amputatus* Say, and *Amara carinata* (LeConte), were chosen for weed seed feeding assays to determine weed seed preference and consumption. These species were selected based on their higher abundance during the 2012 field season. The beetles were hand-collected from sugar beet research fields at the Mitchell research farm during their respective peak abundances. For this reason, all species were not tested at the same time. Prior to starting the experiment, all test insects were offered de-hulled millet seed (*Panicum miliaceum* L.) to ensure that they would accept food. Thereafter, they were provided with moisture but starved for 24 hours before the experiment was initiated.

Feeding assays were conducted under choice conditions only, where ground beetles were presented a choice between the seeds of the same weed species used in the field experiment outlined above (yellow foxtail, barnyardgrass, kochia, and lambsquarters). Individuals were enclosed in plastic petri dishes (100 x 15 mm) containing a damp cotton wick as a source of

moisture. A single beetle was introduced into each enclosure and presented with a total of 50 seeds from each weed species. Seed densities were selected based on a preliminary study where seed consumption was monitored over 48 h in order to ensure that the supply of seeds would not be exhausted during the experiment. The beetles were allowed 48 h to feed during the bioassay before they were collected and frozen at -20 °C, dried at room temperature, and their mass recorded to the nearest 0.0001 g. The proportion of seeds destroyed per beetle for each weed species was recorded if seeds were cracked or visibly chewed upon. The assays were conducted in a growth chamber (27 °C, 16:8 [L:D]).

After the data were fitted to a Poisson distribution, the mean number of seeds consumed of each weed species by a given ground beetle species was compared by means of a one-way ANOVA. Significantly different means were separated using Tukey's HSD multiple comparison ad hoc procedure at the  $\alpha = 0.05$  level of significance in SAS version 9.2 (SAS 2008). Preference for the seeds of either broad-leaf weeds (lambsquarters and kochia) or grass weeds (barnyardgrass and yellow foxtail) were also tested for the four ground beetle species by using orthogonal comparisons.

**Field predation rates:** To test for differences in the rate of pest removal between the two tillage systems, as well as any differences in pest removal during different times of the day, a field predation study was conducted concomitant to the weed seed removal study. This experiment had a 2 x 2 factorial treatment design. Tillage (two levels: conventional and zone tillage in a RCBD) and time of day (two levels: day and night) constituted the two main factors. Waxworm larvae (*Galleria mellonella* L.) were used as surrogate prey. Because the weed seed removal experiment and the field predation experiment were conducted on approximately the same dates, the prey-removal study was also repeated three times throughout both 2012 (19-20 July, 15-16 August, and 14-15 September) and 2013 (18-19 July, 15-16 August, and 18-19 September). Following the protocol of Lundgren *et al.* (2006), waxworm larvae (of

approximately equal size) were pinned onto triangular clay bases (Original Sculpey® oven-bake clay) with #2 insect pins (Bioquip® insect pins) to prevent their escape. The larvae were placed into the same vertebrate exclusion cages used in the post-dispersal weed seed removal experiment, with the clay bases buried below soil level. Three larvae were enclosed in each cage. Because predator activity can differ markedly between different times of the day due to the presence of nocturnal, diurnal, and crepuscular species, prey removal was monitored for 24 hours from 07:00 am – 06:00 am. The first observation period took place from 07:00 am to 06:00 pm (day), while the second lasted from 07:00 pm to 06:00 am (night). Larvae that were removed or killed were recorded as preyed upon. Additionally, larvae with visible chewing scars, but which were still alive, were also scored as having been preyed upon. Any predators observed feeding on the larvae at the time of sampling were collected for further identification.

Predation of waxworm larvae was low during the first and last sampling dates of both years; therefore, the data from each exclusion cage were pooled across sampling dates for the total number of larvae removed per cage within each year ( $n = 9$ ). Data for the two years were analyzed separately by means of a two-way ANOVA using the PROC GLIMMIX procedure in SAS version 9.2 (SAS 2008). The data were fitted to a binomial distribution which allowed for comparing the proportion of larvae removed between tillage practices and time of day. Significantly different means between tillage and time of day were separated using a protected LSD ad hoc test at the  $\alpha = 0.05$  level of significance.

**Weed seed age preference assay:** An experiment was conducted to determine whether one dominant ground beetle species showed preference for old (> 5 years old) versus fresh (collected from the parent plant in the current year of study) weed seeds. *Harpalus pensylvanicus* was selected, because it was the most active ground beetle species observed at the time of seed rain (mid- to end September 2013). Individual beetles were hand-collected from a sugar beet field and offered de-hulled millet seed to ensure that they would accept food. Thereafter, the beetles



were starved for a period of 24 hours while being provided a moistened cotton wick. Beetles were then enclosed in plastic petri dishes (100 x 15 mm) containing a damp cotton wick for moisture. A single beetle was introduced into each enclosure and presented with a total of  $n = 50$  seeds from each age group of either barnyardgrass or lambsquarters seeds ( $n = 24$  beetles for each weed species). Enough seeds were included to prevent the beetles from consuming all the seeds of a particular age. The seeds from the two age groups were mixed before being placed into the feeding arenas. Barnyardgrass and lambsquarters were chosen because one was a grass and the other a broad-leaf weed, their high abundance in the area of research, difference in seed size between the two species, and because they seemed to be the most preferred weed species by *H. pensylvanicus* in the choice feeding assay described above. The beetles were allowed 30 hours to feed on the seeds before the experiment was terminated. This study was conducted under controlled circumstances using insect growth chambers (25 °C, 12 L: 12 D).

The mean number of weed seeds consumed for the two different age groups within each weed species was compared with a one-way ANOVA after being fitted to a Poisson distribution. Significantly different means were separated by means of a protected LSD ad hoc procedure at the  $\alpha = 0.05$  level of significance in SAS version 9.2 (SAS 2008).

## Results

**Beneficial arthropod activity density:** During 2012, the average soil surface residue was 8.2 and 81.2% in the conventional- and zone-tillage plots, respectively. In 2013, 4.9 and 70.5% surface residue was measured in the conventional- and zone-tillage plots, respectively. A total of 5,831 and 3,783 individual beneficial arthropods were sampled during the 2012 and 2013 growing seasons, respectively (Table 5.1). Due to the low abundance of harvestmen ( $n = 66$  individuals for both years combined) and coccinellids ( $n = 28$  individuals for both years), these taxa were not considered for any further analyses. Carabidae and Staphylinidae comprised 78% of

the total beneficial arthropod abundance during the 2012 cropping season, and 74% during 2013 season.

The yearly mean number of ground beetles collected over the six sample dates in each tillage type is presented in Figure 5.1 (a and b). A two-way ANOVA with repeated measures revealed that ground beetle activity density was similar between the two tillage practices during both years (2012:  $F_{1,4} = 0.77$ ,  $P = 0.43$ , and 2013: tillage:  $F_{1,13} = 0.34$ ,  $P = 0.57$ ). However, a significant effect of sampling time was observed during both seasons (2012:  $F_{5,45} = 52.01$ ,  $P < .001$ , and 2013:  $F_{5,36} = 18.39$ ,  $P < .001$ ). The peak activity for both 2012 ( $41.85 \pm 5.63$  beetles per trap) and 2013 ( $16.79 \pm 1.50$  beetles per trap) seasons occurred on the fifth sampling date in August. The interaction between tillage practice and sampling time was non-significant (2012:  $F_{5,45} = 1.27$ ,  $P = 0.29$ , and 2013:  $F_{5,36} = 1.21$ ,  $P = 0.32$ ). Ground beetle activity density was lower during 2013.

Both tillage practice (2012:  $F_{1,17} = 24.02$ ,  $P < .001$ , and 2013:  $F_{1,7} = 10.07$ ,  $P = 0.01$ ) and time (2012:  $F_{4,13} = 13.91$ ,  $P < .001$ , and 2013:  $F_{4,46} = 4.28$ ,  $P = 0.01$ ) had a significant influence on centipede activity density during both years (Figures 5.1 c and d). Furthermore, a significant interaction between tillage practice and time was measured for both years (2012:  $F_{4,13} = 4.77$ ,  $P = 0.01$ , and 2013:  $F_{4,46} = 3.53$ ,  $P = 0.01$ ). During both years, centipede activity densities were similar in both tillage systems up to the third sampling dates (05 July 2012 and 10 July 2013), but their activity was almost always significantly higher in the zone tillage treatment after this.

Spider activity density differed between the two tillage practices (2012:  $F_{1,5} = 31.03$ ,  $P = 0.002$ , and 2013:  $F_{1,9} = 11.20$ ,  $P = 0.008$ ) (Figures 5.1 e and f). During both years, their activity was higher in the zone tillage plots (2012:  $4.27 \pm 0.33$  versus  $2.33 \pm 0.21$  spiders/trap, and 2013:  $4.07 \pm 0.32$  versus  $2.73 \pm 0.24$  spiders/trap). Their activity was also significantly impacted by sampling time in both years with an increase and subsequent decrease in their abundance as the

season progressed (2012:  $F_{5,52} = 14.97$ ,  $P < .001$ , and 2013:  $F_{5,31} = 29.69$ ,  $P < .001$ ). However, their abundance peaked during the fifth sampling date in 2012 ( $5.86 \pm 0.72$  spiders/trap), but earlier on the third sampling date in 2013 ( $7.70 \pm 0.68$  spiders/trap). For this taxon, there was no tillage by sampling time interaction (2012:  $F_{5,52} = 1.64$ ,  $P = 0.17$ , and 2013:  $F_{5,31} = 0.27$ ,  $P = 0.93$ ).

The mean number of rove beetles collected was marginally impacted by both tillage (2012:  $F_{1,11} = 43.14$ ,  $P < .001$ , and 2013:  $F_{1,11} = 3.91$ ,  $P = 0.07$ ) and sampling time (2012:  $F_{5,141} = 17.81$ ,  $P < .001$ , and 2013:  $F_{5,54} = 4.92$ ,  $P = 0.001$ ) (Figure 5.1 g and h). There was also a significant interaction between tillage and sampling time (2012:  $F_{5,141} = 2.29$ ,  $P = 0.05$ , and 2013:  $F_{5,54} = 2.30$ ,  $P = 0.06$ ). With the exception of the first and fifth sampling dates (24 May and 14 August), rove beetle activity was significantly higher in the zone tillage plots during 2012. The interaction was due to high beetle activity in the zone tillage treatment on July 29. During 2013, differences in rove beetle activity density between tillage systems were observed on the fourth and fifth sampling dates (30 July and 21 August). Their numbers remained generally constant in the conventional tillage plots.

**Ground beetle species richness and diversity:** With 5,421 total specimens (Table 5.1), ground beetles were the most commonly collected beneficial arthropods, comprising 41 species in 19 genera (Table 5.2). However, their numbers and species diversity were notably lower during 2013. Few ground beetle species dominated the samples; *H. erraticus* Say, *Elaphropus anceps* (LeConte), *H. pensylvanicus*, *Amara carinata* (LeConte), *Cicindela punctulata punctulata* Olivier, *Bembidion quadrimaculatum oppositum* Say, and *H. amputatus* comprising ca. 90% of the total diversity in 2012 (Table 5.2). These same species, with the exception of *H. amputatus* and the addition of *B. tetracolum tetracolum* Say, *Clivina impressifrons* LeConte, and *Stenolophus comma* (Fabricius), comprised ca. 90% of the total ground beetle captures during

2013 (Table 5.2). Of these, *H. erraticus* and *E. anceps* made up 67% of this abundance in 2012, and 54% in 2013. *Harpalus erraticus* was especially numerous in the first year of the study.

During both 2012 and 2013, there was a significant effect of sample time on ground beetle species richness (Table 5.3). The effect of tillage on species richness was significant during 2012 (more species sampled in the zone tillage plots), but not during 2013. In both years no interaction between sample date and tillage was observed (2012:  $F_{5,40} = 1.80$ ,  $P = 0.14$ , and 2013:  $F_{5,40} = 0.61$ ,  $P = 0.69$ ). Sample time significantly impacted the Simpson's diversity in both years, but the tillage effect was only significant during 2012 (with higher diversity under the zone tillage treatment). No interaction between these effects was measured (2012:  $F_{5,40} = 1.72$ ,  $P = 0.15$ , and 2013:  $F_{5,40} = 0.95$ ,  $P = 0.46$ ). Lastly, Simpson's evenness was also significantly impacted by sampling date during both years, but not by tillage, with no observed interaction (2012:  $F_{5,40} = 1.03$ ,  $P = 0.41$ , and 2013:  $F_{5,40} = 1.37$ ,  $P = 0.26$ ) (Table 5.3).

When comparing the most dominant species, it was evident that some species preferred the zone tillage system, while others were more abundant under the conventional tilled system (Table 5.4). Most of the observed preferences, however, were not consistent between years. However, in both years, *H. pensylvanicus* showed higher activity in the zone tillage plots.

**Post-dispersal weed seed removal from the field:** The recovery rate from control cages during 2012 was: 96.89% kochia, 99.67% yellow foxtail, and 100% for both barnyardgrass and lambsquarters in the conventional tillage plots. In the 2012 zone tillage plots, the recovery rates were: 99% yellow foxtail, 99.33% barnyardgrass, 99.56% lambsquarters, and 100% kochia. The recovery rate from control cages during 2013 was: 98.67% kochia, 99.33% yellow foxtail, 99.67% barnyardgrass, and 100% lambsquarters in the conventional tillage treatments. In the 2013 zone tillage plots, the recovery rates were: 94.67% kochia, 98.44% lambsquarters, and

100% for both yellow foxtail and barnyardgrass. Due to these high recovery rates there were no calculated corrections used for seed loss from sources within the exclusion cages.

During 2012, the proportion of weed seeds consumed differed significantly between tillage practices ( $F_{1,4} = 10.62$ ,  $P = 0.03$ ) and weed species ( $F_{3,24} = 5.48$ ,  $P = 0.005$ ), with no observed interaction ( $F_{3,24} = 0.84$ ,  $P = 0.49$ ). A higher mean proportion of weed seeds were consumed in zone tillage treatments ( $0.39 \pm 0.05$ ) compared to the conventional tillage treatments ( $0.19 \pm 0.03$ ) (Figure 5.2). Furthermore, a significantly higher proportion of barnyardgrass was consumed compared to both yellow foxtail ( $t = 2.92$ ,  $P = 0.008$ ) and lambsquarters ( $t = 3.66$ ,  $P = 0.001$ ), while the consumption of kochia was higher compared to that of lambsquarters ( $t = 2.47$ ,  $P = 0.02$ ) (Figure 5.2).

During the 2013 field season, there was a significant tillage x weed species interaction, whereas the effect for tillage was marginally significant; weed seed consumption in the conventional tilled plots approached the consumption levels observed in the zone tillage plots (tillage:  $F_{1,4} = 8.02$ ,  $P = 0.05$ ; weed species:  $F_{3,24} = 9.14$ ,  $P < .001$ ; tillage x weed species:  $F_{3,24} = 3.62$ ,  $P = 0.03$ ; Figure 5.3). The interaction was due to the significantly higher consumption of kochia in the zone tillage treatment compared to the conventional tillage treatment ( $t = -4.16$ ,  $P < .001$ ) (Figure 5.3). Especially under the zone tillage treatment, the consumption rates of some of the weed species were notably high. Under this tillage treatment, 60% of the kochia seeds were consumed, while 54% of the barnyardgrass seeds were consumed.

**Weed seed choice feeding assays:** Weed species had a significant effect on the number of seeds consumed for all four beetle species (*A. carinata*:  $F_{3,33} = 25.55$ ,  $P < .001$ ; *H. amputatus*:  $F_{3,33} = 36.21$ ,  $P < .001$ ; *H. erraticus*:  $F_{3,33} = 47.41$ ,  $P < .001$ ; *H. pensylvanicus*:  $F_{3,33} = 91.45$ ,  $P < .001$ ; Table 5.5). Ground beetles preferred to consume significantly more lambsquarters seeds than other weed species (16 versus 5 or less: Table 5.5). Both *A. carinata* and *H. amputatus*,

preferred to consume broadleaves (kochia and lambsquarters) over grasses. In contrast, *H. erraticus* and *H. pensylvanicus* both had barnyardgrass seeds as the second most consumed weed species, albeit not statistically different from kochia in the case of *H. erraticus*. For all four beetle species tested, the mean number of broadleaf weed seeds consumed (lambsquarters and kochia) was significantly higher ( $P < 0.05$ ) compared to the grassy weeds (barnyardgrass and yellow foxtail), owing to the high consumption rate of lambsquarters (as determined by an orthogonal test). Averaged over all weed species, *H. erraticus* consumed an average of 4.94 seeds per individual per day, *H. pensylvanicus* 4.44 seeds per individual per day, *H. amputatus* 2.51 seeds per individual per day, and *A. carinata* 1.66 seeds per individual per day. Therefore, the overall number of weed species consumed by each of the four beetle species was not necessarily related to their size (dry weight). Although the heaviest species, *H. erraticus*, consumed the most weed species, *H. amputatus* (with the lightest recorded dry weight) consumed more seeds compared to *A. carinata*.

**Field predation rates:** During 2012, the proportion of waxworm larvae removed from the exclusion cages differed significantly between the time of day, but not between tillage practices. There was no interaction between tillage or time of day (tillage:  $F_{1,4} = 0.01$ ,  $P = 0.92$ ; time of day:  $F_{1,8} = 36.56$ ,  $P < .001$ ; tillage x time of day:  $F_{1,8} = 1.28$ ,  $P = 0.29$ ). A higher proportion of larvae were consumed during the night compared to the day (Figure 5.4). During the 2013 growing season, time of day had a significant impact on the proportion of larvae removed (tillage:  $F_{1,4} = 0.12$ ,  $P = 0.74$ ; time of day:  $F_{1,8} = 52.53$ ,  $P < .001$ ; tillage x time of day:  $F_{1,8} = 1.03$ ,  $P = 0.34$ ). As in the case of the 2012 growing season, the proportion of waxworm larvae consumed was highest at night (Figure 5.5).

Despite the level of apparent predation in the field, few predatory arthropods were observed feeding on waxworm larvae. During 2012, only eight predatory arthropods were collected that fed on the larvae, including two individuals of the same species in the genus

*Geocoris* (Hemiptera: Geocoridae), five ground beetles (Coleoptera: Carabidae), and one harvestman (Opiliones). The ground beetle species observed were *H. amputatus* (three individuals), *H. pensylvanicus* (one individual), and one unidentified species. During 2013, a total of eight predatory arthropods was also observed feeding on the waxworm larvae, but, in addition to these, seven waxworm larvae were observed being attacked by *Tetramorium caespitum* (Hymenoptera: Formicidae). Of the eight predators observed, one was a true bug, *Peritrechus convivus* (Stål) (Hemiptera: Rhyparochromidae), while ground beetles composed the remainder. The ground beetle species collected were two individuals each for *E. anceps*, *B. quadrimaculatum*, and *H. erraticus*, and one individual of *A. carinata*.

**Weed seed age preference assay:** *Harpalus pensylvanicus* did not have a preference for seed age of barnyardgrass ( $F_{1,23} = 1.68$ ,  $P = 0.21$ ) (Figure 5.6) with a mean consumption of  $6.80 \pm 0.78$  fresh seeds and  $7.79 \pm 0.87$  old seeds. However, this species did show a preference for old lambsquarters seeds ( $F_{1,23} = 104.34$ ,  $P < .001$ ) consuming a mean of  $17.38 \pm 1.91$  fresh seeds vs.  $31.44 \pm 3.31$  old seeds.

## Discussion and Conclusions

The positive impact of epigeal natural enemies on pest insect populations in sugar beet agroecosystems has been reported previously (Hull and Gates 1953; Dunning *et al.* 1975; Landis and Van der Werf 1997). Therefore, there are advantages in adopting farming practices that conserve and enhance beneficial arthropods in sugar beet (Kendall 2003). The interaction of tillage and arthropod conservation has received much attention (reviewed by Kendall 2003). Past studies comparing the effects of reduced-tillage methods on beneficial epigeal arthropods have provided contradicting results, which seems to be related to the group/species being compared. Research has shown increased abundance for certain taxa and/or species (e.g. House and Parmelee 1985; House 1989; House *et al.* 1989; Rice and Wilde 1991; Cárcamo 1995; Baguette

and Hance 1997; Clark *et al.* 1997; Wilson-Rummenie *et al.* 1999; Langmaack *et al.* 2001; Holland and Reynolds 2003, Witmer *et al.* 2003; Sharley *et al.* 2008), while others saw decreased abundance (Rice and Wilde 1991; Baguette and Hance 1997; Clark *et al.* 1997; Holland and Reynolds 2003; Sharley *et al.* 2008) or no differences at all (Stinner and McCartney 1988; Rice and Wilde 1991; Cardina *et al.* 1996; Krooss and Schaefer 1998). However, in general, most have reported decreased abundance and activity density of beneficial epigeal arthropods in response to increased levels of cultivation.

In this study, centipede, spider, and rove beetle populations were mostly favored by the zone tillage system. Two plausible explanations for their higher activity densities under the zone tillage system could be attributed to the indirect effects of conventional tillage, such as decreased prey availability (and, conversely, more prey being available in the reduced tillage system), or improved micro habitat (with more protection from intraguild predation) in the zone tillage system as a result of the higher percentage of crop residue. Decreased abundance of these taxa in response to plowing (and other forms of disturbance cultivation) has been reported elsewhere (Blumberg and Crossley 1983; Stinner and McCartney 1988; Krooss and Schaefer 1998; Cromar *et al.* 1999; Holland and Reynolds 2003; Sharley *et al.* 2008). For example, tillage has been shown to reduce Collembola populations significantly (Hendrix *et al.* 1986; Stinner and McCartney 1988; Miyazawa *et al.* 2002; Petersen 2002), a detritivorous group that constitutes an important component in the diets of generalist natural enemies (Blide *et al.* 2000; Petersen 2002). Higher organic matter on the soil surface resulting from crop residue in the zone tillage plots would likely support higher detritivore populations, thereby increasing predator abundance (House and Parmelee 1985). Reduced tillage methods, as compared to inversion plowing, are also less likely to cause emigration of epigeal beneficial arthropods (Thorbek and Bilde 2004). The increased crop residue in zone tillage systems not only improves the habitable environment, but also alters the microclimatic conditions (e.g. soil humidity) to the possible benefit of these three



taxa and their prey by preventing desiccation. Indeed, it is widely reported that increased organic residue on the soil surface, enhances beneficial arthropod abundance in agroecosystems (Clark *et al.* 1993; Brust 1994; Miñarro and Dapena 2003; Thomson and Hoffmann 2007). Finally, direct mortality resulting from inversion plowing, could also have contributed to decreased activity of centipedes, spiders, and rove beetles under this tillage system.

This study highlights the fact that generalist beneficial arthropod activity density for all taxa varies throughout the season. The implication of this varied and temporally-dispersed activity is that the combined activity of all taxa may ensure season-long pest control of various life stages of a range of insect pests (Kendall 2003). It assures that some beneficial taxa will be present and have a regulatory impact on pest species during their immigration and establishment before specialists can respond numerically (Janssens and De Clerq 1990; Holopainen and Helenius 1992; Landis and Van der Werf 1997). It is the diversity of species, coupled with their generalist feeding habits, which potentially makes generalist natural enemies important in pest management. This is despite the fact that the impact of any single beneficial species on pest populations might be limited, or the fact that the abundance of individual beneficial species may vary between years (Kendall 2003).

The impact of tillage regime on the activity density of ground beetles was negligible. Two possible explanations for this can relate to ground beetle dispersal capabilities or the constraints of pitfall sampling. Ground beetles are excellent dispersers (Wallin and Ekbom 1988), and they may have readily moved between plots compared to other taxa. Pitfall trap captures measure the activity density of ground-dwelling invertebrates and not absolute density. Ground beetle abundance could be lower in the conventional tilled plots, but increased movement under this system could have enhanced the rate of capture (Shearin *et al.* 2007). Both scenarios could have led to the equal abundance observed between the two tillage regimes. However, it has been observed that some ground beetle species are favored by plowing while others are impacted

negatively (e.g. House 1989; Cárcamo 1995; Clark *et al.* 1997; Holland and Reynolds 2003). Menalled *et al.* (2007) reported that the total activity density of ground beetles was higher in a conventional tilled system (comprising moldboard plowing), but the activity density of weed seed consumer species was higher in their no-till systems compared to the conventional tilled system. Shearin *et al.* (2007) also reported that moldboard plowing reduced granivorous ground beetle activity density significantly, while a predatory ground beetle species was negatively affected by all tillage systems investigated. In his review on the influence of tillage on epigeal predatory arthropods, Kendall (2003) concluded that because dominant ground beetle species often react differently to conventional and reduced tillage systems, little or no differences in their activity density between these tillage types could be detected at the family level. This is a plausible explanation for a lack of difference in ground beetle activity density observed in this study. Because of this, and as recommended by both Barney and Pass (1986a) and Kendall (2003), abundant and functionally important taxa, such as ground beetles, need to be examined on the species level, rather than on the family level due to their differing feeding specializations and habitat preferences.

Focusing on the species level for ground beetles, it was clear that only a few species dominated numerically (comprising ca. 90% of all captured individuals). Many of the most abundant species sampled in this study have the capacity to consume both prey and weed seeds as shown with both field and growth chamber observations, highlighting their importance to contributing to the sustainable management of these pests (e.g. Barney and Pass 1986b; Cardina *et al.* 1996). Furthermore, some species actually showed a preference for the zone tillage system, some preferred the conventional tillage system, while many remained unaffected. However, with the exception of *H. pensylvanicus*, their preference for a particular tillage system was not always detectable between years. Examining ground beetles on the species level also revealed that both species richness and their diversity was affected by tillage regime, as was evidenced by the

significant differences in these two parameters between the cultivation practices during 2012. Furthermore, the significant effect of sampling time on all three indices during both years indicates that the species assemblage and activity density of the different species will also vary as the season progress, in addition to their changing activity density.

Increased crop residue left on the soil surface following cultivation has been shown to increase post-dispersal weed seed removal (Brust and House 1988; House and Brust 1989; Menalled *et al.* 2007). Such observations indicate that these environments provide shelter for beneficial arthropods (Reader 1991), and that reduced soil disturbance, also favor these organisms. Furthermore, there is evidence that the type of residue left on the soil surface can impact the number of weed seeds consumed; post-dispersal weed seed removal is highest in plots with corn residue, compared to residue from other crops, such as wheat stubble (Cromar *et al.* 1999). The results of this study agree with these findings as evidenced by higher post-dispersal weed seed removal rates in the zone tillage plots. In addition, selection between seeds from different weed species under both field and laboratory conditions has been shown with this study. Similar findings have been widely reported (e.g. Jorgenson and Toft 1997; Tooley *et al.* 1999; Honek and Jarosik 2000; Honek and Martinkova 2001; Honek *et al.* 2003; Honek *et al.* 2005; Heggenstaller *et al.* 2006; Lundgren *et al.* 2006; Klimeš and Saska 2010; Meiss *et al.* 2010).

Under field conditions, barnyardgrass and kochia were favored most by granivores (both seasons), demonstrating the capacity of these organisms to contribute to the destruction of both grass and broad-leaf weed seeds. With the exception of kochia, the few differences in the consumption rate for the remaining weed species' seeds between the two tillage practices during 2013 might be a function of lower ground beetle activity density recorded during this season. Alternatively, reduced food choice, combined with greater mobility under plowed condition (House and All 1981; Crist *et al.* 1992), could also have contributed to this phenomenon. Cardina

*et al.* (1996) also observed that the rate of post-dispersal weed seed removal can vary between seasons.

Ground beetles are renowned for their ability to consume weed seeds, forming either a major component of their diets, or a sporadic source of nourishment (Johnson and Cameron 1969). Due the use of vertebrate exclusion cages, this study only investigated post-dispersal weed seed removal by arthropods. It is, however, important to note that other organisms, such as rodents, could potentially also consume a large proportion of weed seeds under field conditions (e.g. Brust and House 1988; Cardina *et al.* 1996; Harrison *et al.* 2003). No signs (e.g. droppings or the actual presence) of rodents were detected within the exclusion cages used in this study, suggesting that the cages worked well in their exclusion, and that weed seed removal could be attributed to the action of arthropods. Furthermore, the pitfall samples showed very low (or none) numbers of other important weed seed feeders in other systems, such as crickets, isopods, ants, and slugs (Mittelbach and Gross 1984; Cardina *et al.* 1996; Hurst and Doberski 2003; O'Rourke *et al.* 2006; Honek *et al.* 2009). None of these groups were observed feeding on the seeds in our seed choice tests in the field. However, several ground beetle species were regularly observed feeding within the exclusion cages on the weed seeds and they constituted a majority of total pitfall samples; therefore they likely constitute the key invertebrate seed feeders in sugar beets as noted for other systems (Brust and House 1988; Cromar *et al.* 1999; Honek *et al.* 2003; Westerman *et al.* 2003).

Seed choice in the laboratory assay differed from that observed in the field. Common lambsquarters was the most preferred weed species for all granivorous ground beetle species under controlled conditions. Possible explanations for this difference could be that only four ground beetle species were tested in the growth chambers, while the granivorous arthropod fauna under field conditions is composed of many species with a wide array of food items. Furthermore, Klimeš and Saska (2010) observed differences in weed seed choice both within and between the

larval and adult stages of ground beetle species and indicated that larval ground beetles might be more important weed-seed consumers than adults. This study did not determine the importance of adult ground beetles versus that of ground beetle larvae in weed seed consumption, because larvae were rarely observed. Adis (1979) noted that pitfall traps would underestimate larval abundance as a result of larval size and movement speed. Lastly, Lundgren *et al.* (2006) showed that actual seed feeder densities, measured using quadrature samples, yielded a better representation of the biological control potential of these organisms, compared to measuring their activity density by means of pitfall trapping. This is probably due to the constraints associated with pitfall trapping mentioned earlier.

In the laboratory choice feeding assay, it was evident that the larger ground beetle species, *H. pensylvanicus* and *H. erraticus*, were more efficient consumers of the larger seeds of barnyardgrass compared to the two remaining smaller ground beetle species. Schoener (1971) reported that seed feeders would feed on the largest seeds they can handle. In contrast, the smaller size of lambsquarters did not prohibit their destruction by these relatively large ground beetle species, and it has been shown by Lundgren and Rosentrater (2007) that ground beetle species, such as *H. pensylvanicus*, actually preferred smaller tougher seeds over larger seeds with weaker coats (probably due to factors related to nutritional pay-offs).

In contrast with the situation observed for weed seed consumption, the number of surrogate prey (waxworm larvae) preyed upon did not differ between the tillage practices. This was despite the fact that pure predatory groups such as spiders and (possibly) centipedes had a higher abundance in the zone tillage plots. Because the larvae used in this study were relatively large (compared to the size range for most of the collected predatory arthropods), combined with the fact that they were frequently either partially or totally consumed, suggest that larger predators might have been responsible for their consumption. Larger predatory arthropods, especially ground beetles which are known to be good dispersers (Wallin and Ekbohm 1988),

would find it easier to move between relatively smaller plots; possibly contributing to the lack of observable differences between tillage systems. As an example, the relatively large *H. erraticus* did not exhibit any differences in activity density between the tillage practices. The fact that night-time predation rates were higher is to be expected, considering that many predatory arthropods are nocturnal (e.g. Vickerman and Sunderland 1975).

Considering the tremendous amount of seeds that can be produced by certain weed species, it is reasonable to expect that many seeds enter the seed bank where they will not be immediately consumed. The question then arises whether or not seed feeding arthropods will preferentially consume older seeds exposed on the soil surface? This study indicated that *H. pensylvanicus* has no preference for seed age for some weed species (e.g. barnyardgrass), while the reverse is true for others (e.g. lambsquarters). Two possible explanations why *H. pensylvanicus* chose old over fresh lambsquarter seeds might be related to seed toughness and strength and/or phytochemical seed protection. Lundgren and Rosentrater (2007) established that *H. pensylvanicus* preferred to feed on tougher and denser seeds (lambsquarters) under choice conditions. It is to be expected that seeds will become denser with age as a result of dehydration, thereby influencing their choice for these seeds. Fresh lambsquarter seeds used in this study still had their outer softer seed coats attached, while these were not present on the older seeds. The seed coat might afford protection against granivory after they are shed from the parent plant, possibly due to phytochemical properties (Lundgren and Rosentrater 2007). A single ground beetle species (*H. pensylvanicus*) was used in this study, being provided with the seeds from only two weed species. It is possible that other ground beetle species have differing age preferences for weed seeds of various ages.

Based on the results of this study, it can be concluded that there is a strong incentive for adopting reduced tillage practices, such as zone tillage, in western Nebraskan sugar beet agroecosystems. The advent of glyphosate-tolerant sugar beet varieties has facilitated this

adoption. Not only does reduced tillage enhance many of the epigeal beneficial arthropod fauna examined in this study, but it also increases the ability of these organisms to render crucial ecosystem services, such as weed seed consumption. Increased glyphosate applications have led to resistance development in some weed populations, and in this regard, the importance of alternative management strategies, such as conservation biological control, will most likely increase. The importance of examining abundant beneficial arthropods at the species level was also elucidated, as in the case of ground beetles which, on the family level, showed no obvious response to tillage treatments, but they were affected on the species level in terms of their richness, diversity, and evenness of distribution.

**Table 5.1:** Total number of beneficial arthropods (by taxon) collected with pitfall trapping during 2012 and 2013.

Beneficial arthropod taxon	Total number collected	
	2012 <sup>a</sup>	2013 <sup>a</sup>
Araneae (spiders)	702	703
Carabidae (ground beetles)	3,734	1,687
Chilopoda (centipedes)	506	249
Coccinellidae (lady beetles)	24	4
Opiliones (harvestmen)	23	43
Staphylinidae (rove beetles)	842	1,097
Total	5,831	3,783

<sup>a</sup>Total collected over six sampling dates within a particular year ( $n = 360$  pitfall samples/year).



**Table 5.2:** Cumulative number of ground beetle species collected via pitfall trapping over six sampling dates during each field season of 2012 and 2013 in conventional tillage (CT) and zone tillage (ZT) plots.

Species	2012 <sup>a</sup>			2013 <sup>b</sup>		
	CT	ZT	% Total	CT	ZT	% Total
<i>Agonum placidum</i> (Say)	6	5	0.29	7	12	1.13
<i>Amara carinata</i> (LeConte)	82	104	*4.98	24	45	*4.09
<i>Amara cupreolata</i> Putzeys	-	2	0.05	-	-	-
<i>Amara farcta</i> LeConte	6	23	0.78	-	1	0.06
<i>Amara quenseli quenseli</i> (Schönherr)	2	6	0.21	-	-	-
<i>Anisodactylus rusticus</i> (Say)	1	2	0.08	-	-	-
<i>Bembidion nitidum</i> (Kirby)	20	26	1.23	8	28	2.13
<i>Bembidion obscurellum obscurellum</i> (Motschulsky)	25	2	0.72	8	1	0.53
<i>Bembidion quadrimaculatum oppositum</i> Say	58	109	*4.47	61	71	*7.82
<i>Bembidion rapidum</i> (LeConte)	8	48	1.50	10	15	1.48
<i>Bembidion tetracolum tetracolum</i> Say	12	15	0.72	49	19	*4.03
<i>Bradycellus congener</i> (LeConte)	-	-	-	1	-	0.06
<i>Chlaenius tricolor tricolor</i> Dejean	-	2	0.05	2	-	0.12
<i>Cicindela cursitans</i> LeConte	-	1	0.03	-	-	-
<i>Cicindela punctulata punctulata</i> Olivier	101	69	*4.55	19	27	*2.73
<i>Cicindela purpurea audubonii</i> LeConte	1	-	0.03	-	-	-
<i>Clivina impressifrons</i> LeConte	3	2	0.13	27	33	*3.56
<i>Cratacanthus dubius</i> (Palisot de Beauvois)	-	-	-	-	1	0.06
<i>Dyschirius globulosus</i> (Say)	-	2	0.05	-	-	-
<i>Elaphropus anceps</i> (LeConte)	522	393	*24.50	316	213	*31.36
<i>Elaphrus clairvillei</i> Kirby	-	1	0.03	-	-	-
<i>Harpalus amputatus amputatus</i> Say	37	38	*2.01	23	16	2.31
<i>Harpalus caliginosus</i> (Fabricius)	4	3	0.19	-	-	-
<i>Harpalus erraticus</i> Say	1005	575	*42.31	209	175	*22.76
<i>Harpalus herbivagus</i> Say	11	41	1.39	2	10	0.71
<i>Harpalus opacipennis</i> (Haldeman)	1	-	0.03	-	-	-
<i>Harpalus pensylvanicus</i> (DeGeer)	103	162	*7.10	54	119	*10.25
<i>Harpalus reversus</i> Casey	5	12	0.46	3	1	0.24
<i>Harpalus somnulentus</i> Dejean	-	1	0.03	-	-	-
<i>Lebia bivittata</i> (Fabricius)	1	5	0.16	-	-	-
<i>Lebia solea</i> Hentz	1	1	0.05	-	-	-
<i>Microlestes linearis</i> (LeConte)	2	3	0.13	1	5	0.36
<i>Pasimachus elongatus</i> LeConte	-	1	0.03	-	-	-
<i>Poecilus chalcites</i> (Say)	1	-	0.03	-	-	-
<i>Poecilus lucublandus</i> (Say)	1	7	0.21	1	3	0.24
<i>Poecilus scitulus</i> LeConte	4	1	0.13	3	2	0.30

<i>Pterostichus femoralis</i> (Kirby)	-	1	0.03	-	-	-
<i>Pterostichus melanarius melanarius</i> (Illiger)	1	-	0.03	2	2	0.24
<i>Pterostichus permundus</i> (Say)	1	1	0.05	-	2	0.12
<i>Stenolophus comma</i> (Fabricius)	24	20	1.18	44	12	*3.32
<i>Stenolophus conjunctus</i> Say	1	-	0.03	-	-	-
Sum	2,050	1,684	100	874	813	100
Number of species	31	34	39	22	23	25

<sup>a</sup> A total of 3,734 ground beetles collected over six sampling dates.

<sup>b</sup> A total of 1,687 ground beetles collected over six sampling dates.

\* Ground beetle species making up ca. 90% of the total captures within a specified year.

**Table 5.3:** Mean ( $\pm$  SEM) values for species richness, Simpson's diversity, and Simpson's evenness comparisons between the conventional tillage (CT) and zone tillage (ZT) systems during each of the six sampling dates for 2012 and 2013.

		Species richness ( <i>S</i> )		Simpson's diversity ( <i>D</i> )		Simpson's evenness ( <i>E</i> )	
		2012	2013	2012	2013	2012	2013
Tillage:							
	CT	2.82 $\pm$ 0.18	2.41 $\pm$ 0.12	1.93 $\pm$ 0.09	2.08 $\pm$ 0.07	0.72 $\pm$ 0.03	0.85 $\pm$ 0.01
	ZT	3.44 $\pm$ 0.21	2.57 $\pm$ 0.12	2.56 $\pm$ 0.09	2.26 $\pm$ 0.07	0.77 $\pm$ 0.03	0.88 $\pm$ 0.01
	df	1, 4	1, 4	1, 4	1, 4	1, 4	1, 4
	<i>F</i>	10.7	0.93	30	3.25	1.11	4.87
	<i>P</i>	0.03	0.39	0.005	0.15	0.35	0.09
Sample time:							
	1	2.19 $\pm$ 0.22	2.08 $\pm$ 0.19	2.05 $\pm$ 0.17	1.95 $\pm$ 0.12	0.90 $\pm$ 0.03	0.93 $\pm$ 0.02
	2	3.26 $\pm$ 0.28	2.42 $\pm$ 0.20	2.65 $\pm$ 0.17	1.93 $\pm$ 0.12	0.83 $\pm$ 0.03	0.82 $\pm$ 0.02
	3	3.14 $\pm$ 0.27	2.67 $\pm$ 0.21	2.18 $\pm$ 0.17	2.32 $\pm$ 0.12	0.74 $\pm$ 0.03	0.86 $\pm$ 0.02
	4	4.06 $\pm$ 0.32	2.55 $\pm$ 0.21	2.61 $\pm$ 0.17	2.47 $\pm$ 0.13	0.65 $\pm$ 0.03	0.92 $\pm$ 0.02
	5	3.89 $\pm$ 0.31	3.20 $\pm$ 0.23	1.81 $\pm$ 0.17	2.19 $\pm$ 0.12	0.49 $\pm$ 0.03	0.70 $\pm$ 0.02
	6	2.58 $\pm$ 0.24	2.17 $\pm$ 0.19	2.14 $\pm$ 0.17	2.16 $\pm$ 0.13	0.87 $\pm$ 0.03	0.95 $\pm$ 0.02
	df	5, 40	5, 40	5, 40	5, 40	5, 40	5, 40
	<i>F</i>	9.68	3.82	3.56	2.74	50.30	21.32
	<i>P</i>	< .001	0.006	0.01	0.03	< .001	< .001

Values within a column followed by different lower case letters indicate significant differences in ground beetle species richness, Simpson's diversity, or Simpson's evenness between the six individual sampling dates (LSD multiple comparison test,  $\alpha = 0.05$ ).

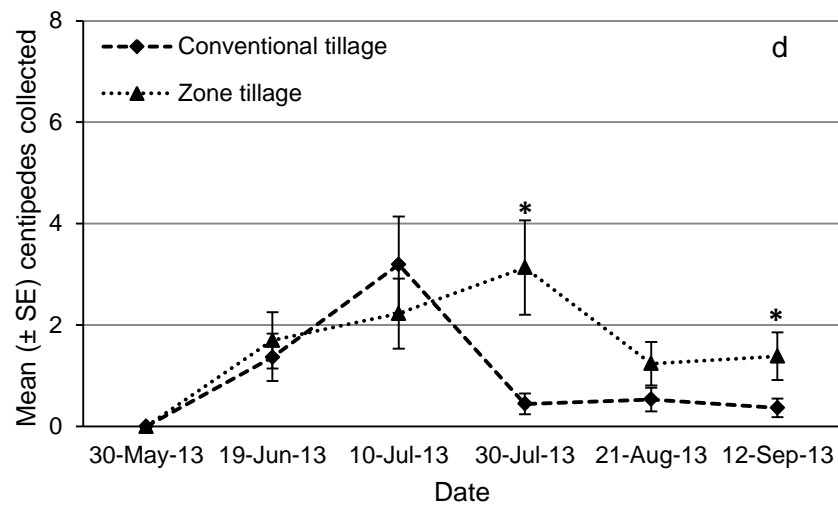
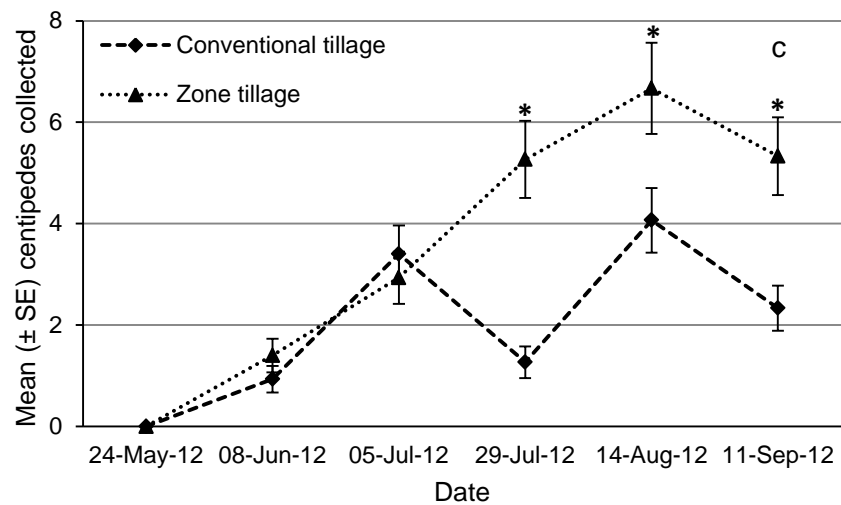
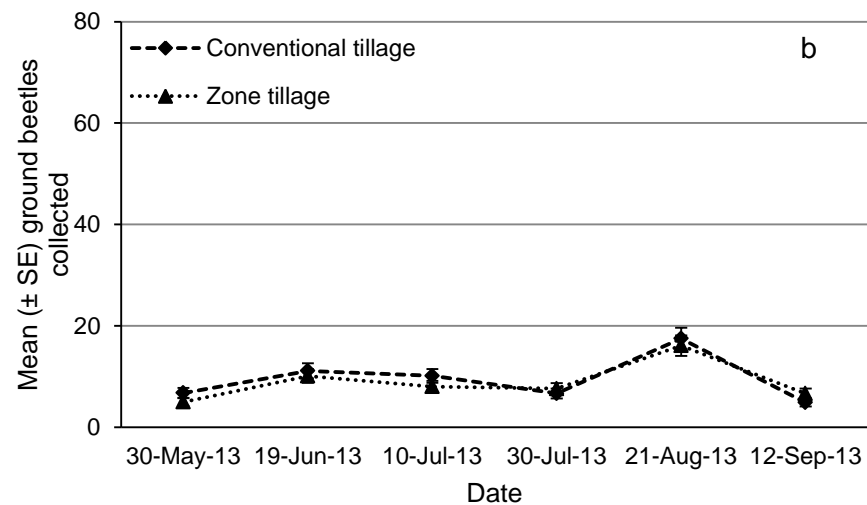
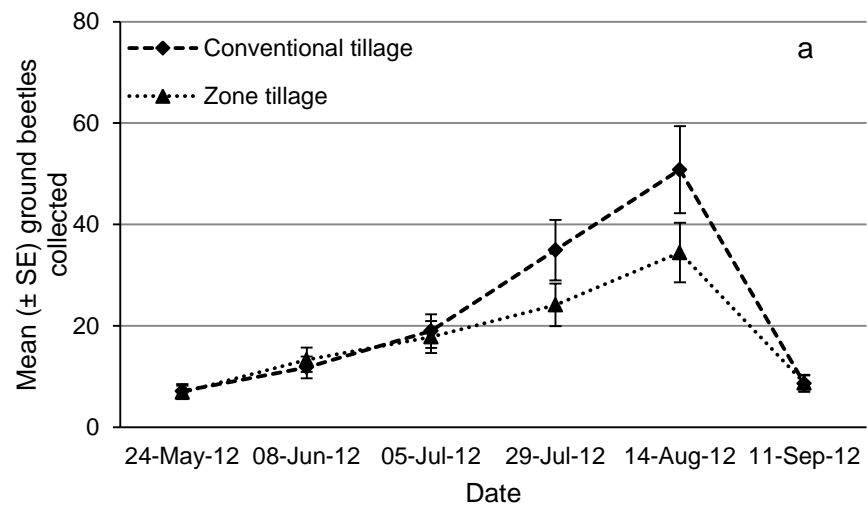
**Table 5.4:** Mean ( $\pm$  SEM) activity density of the most abundant ground beetle species collected by means of pitfall trapping in conventional tilled (CT) and zone tilled (ZT) sugar beets in western Nebraska.

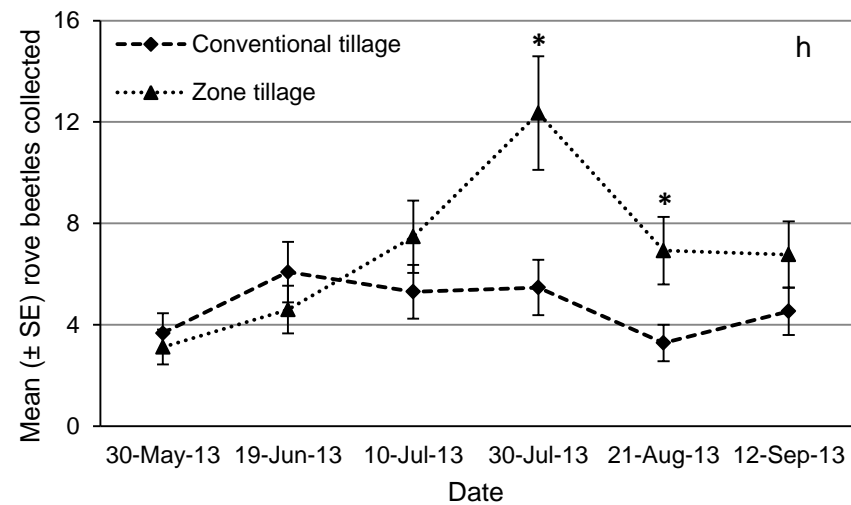
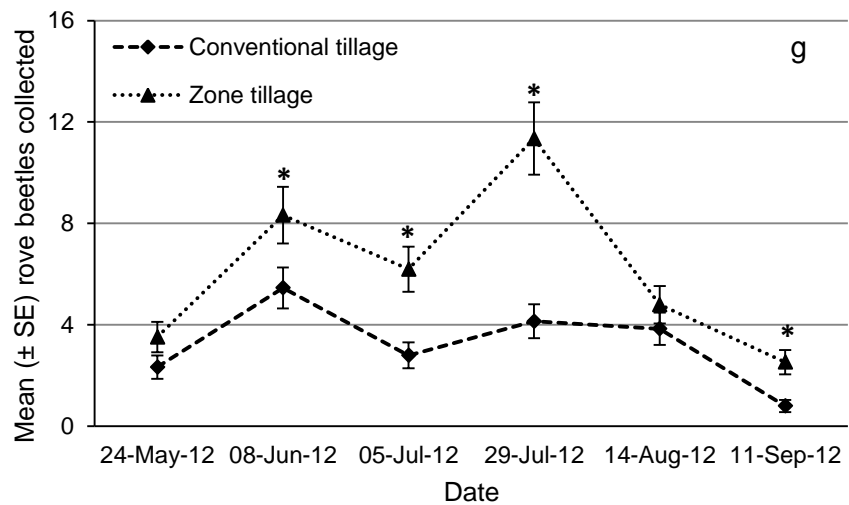
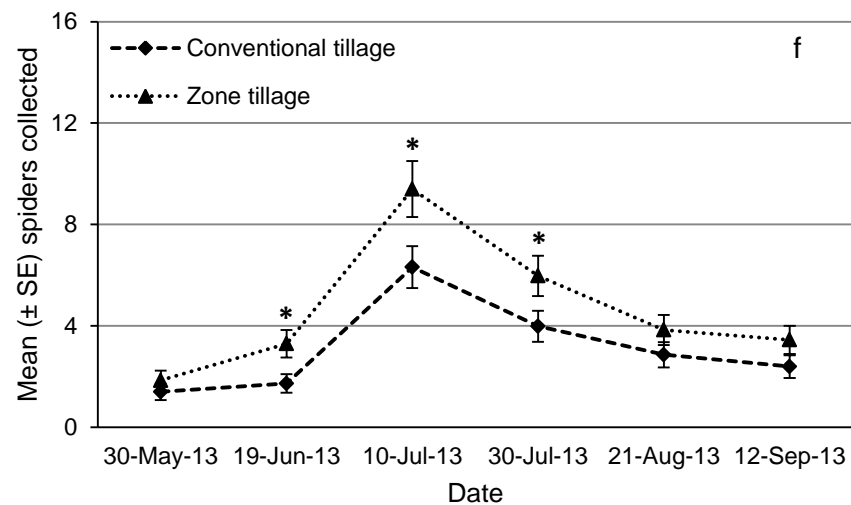
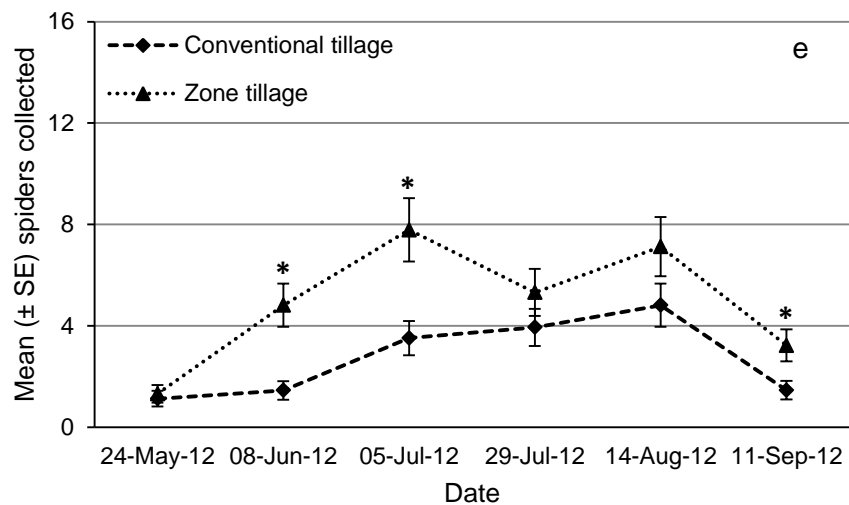
2012	Treatment		df	<i>F</i>	<i>P</i>
	CT	ZT			
<i>Amara carinata</i> (LeConte)	2.62 $\pm$ 0.52	3.35 $\pm$ 0.64	1, 4	0.79	0.42
<i>Bembidion quadrimaculatum oppositum</i> Say	1.93 $\pm$ 0.27	3.63 $\pm$ 0.39	1, 4	12.75	0.02
<i>Cicindela punctulata punctulata</i> Olivier	2.93 $\pm$ 0.86	2.00 $\pm$ 0.60	1, 4	5.95	0.07
<i>Elaphropus anceps</i> (LeConte)	16.90 $\pm$ 2.61	12.46 $\pm$ 1.95	1, 4	3.59	0.13
<i>Harpalus amputatus amputatus</i> Say	1.23 $\pm$ 0.24	1.25 $\pm$ 0.25	1, 4	0.00	0.95
<i>Harpalus erraticus</i> Say	32.34 $\pm$ 6.82	16.69 $\pm$ 3.57	1, 4	4.85	0.09
<i>Harpalus pensylvanicus</i> (DeGeer)	3.43 $\pm$ 0.34	5.40 $\pm$ 0.42	1, 4	12.91	0.02
Other	4.68 $\pm$ 0.80	7.35 $\pm$ 1.20	1, 4	3.64	0.13
2013					
<i>Amara carinata</i> (LeConte)	0.79 $\pm$ 0.19	1.47 $\pm$ 0.29	1, 4	4.63	0.10
<i>Bembidion quadrimaculatum oppositum</i> Say	2.03 $\pm$ 0.26	2.37 $\pm$ 0.28	1, 4	0.76	0.43
<i>Bembidion tetracolum tetracolum</i> Say	1.54 $\pm$ 0.38	0.60 $\pm$ 0.19	1, 4	10.71	0.03
<i>Cicindela punctulata punctulata</i> Olivier	0.62 $\pm$ 0.17	0.88 $\pm$ 0.22	1, 4	1.12	0.35
<i>Clivina impressifrons</i> LeConte	0.90 $\pm$ 0.17	1.10 $\pm$ 0.19	1, 4	0.60	0.48
<i>Elaphropus anceps</i> (LeConte)	10.33 $\pm$ 1.27	6.96 $\pm$ 0.90	1, 4	19.80	0.01
<i>Harpalus erraticus</i> Say	6.83 $\pm$ 0.98	5.67 $\pm$ 0.84	1, 4	0.82	0.42
<i>Harpalus pensylvanicus</i> (DeGeer)	1.79 $\pm$ 0.28	3.96 $\pm$ 0.46	1, 4	16.80	0.01
<i>Stenolophus comma</i> (Fabricius)	1.47 $\pm$ 0.22	0.40 $\pm$ 0.12	1, 4	15.92	0.02
Other	2.35 $\pm$ 0.36	3.73 $\pm$ 0.51	1, 4	5.02	0.09

**Table 5.5:** Mean ( $\pm$  SEM) number of seeds consumed for four weed species by four different granivorous ground beetle species over a 48 h period.

Weed	Ground beetle species				Overall mean
	<i>Amara carinata</i>	<i>Harpalus amputatus</i>	<i>Harpalus erraticus</i>	<i>Harpalus pensylvanicus</i>	
Barnyardgrass	$0.32 \pm 0.16^a$	$0.22 \pm 0.13^a$	$10.26 \pm 1.20^a$	$8.12 \pm 1.26^a$	$4.91 \pm 0.39^a$
Yellow foxtail	$0.06 \pm 0.07^a$	$0.87 \pm 0.29^a$	$0.65 \pm 0.24^b$	$1.25 \pm 0.35^b$	$0.76 \pm 0.13^b$
Kochia	$3.12 \pm 0.87^b$	$6.26 \pm 1.27^b$	$8.06 \pm 1.02^a$	$2.11 \pm 0.48^b$	$5.36 \pm 0.42^a$
Lambsquarters	$6.82 \pm 1.76^c$	$10.20 \pm 1.94^c$	$19.63 \pm 1.96^c$	$21.79 \pm 2.93^c$	$15.78 \pm 0.94^c$
Dry beetle dry weight (mg)	$39.34 \pm 2.21$	$23.02 \pm 1.36$	$85.50 \pm 5.10$	$56.27 \pm 3.35$	

Values within a column followed by different lower case letters indicate significant differences in the mean number of weed seeds consumed by the ground beetle species (Tukey's HSD multiple comparison test,  $\alpha = 0.05$ ).

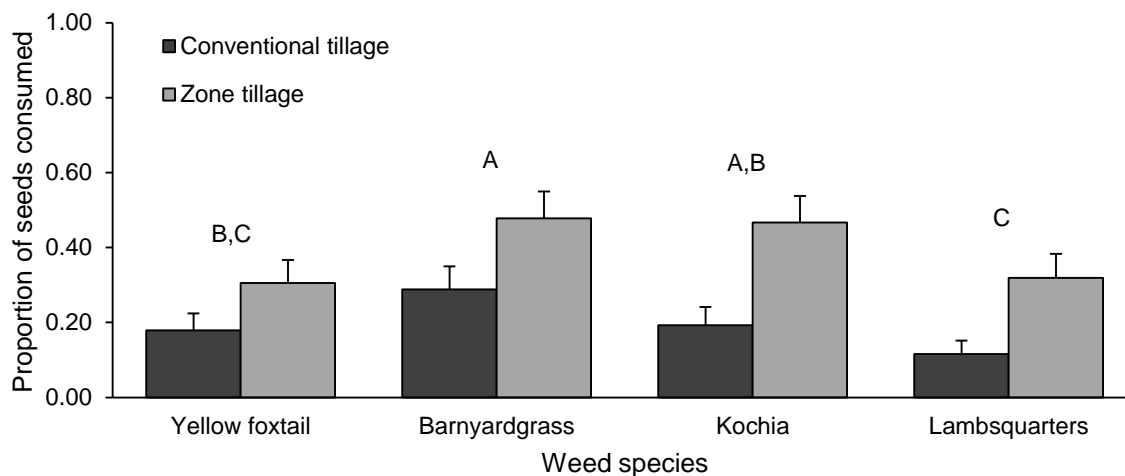




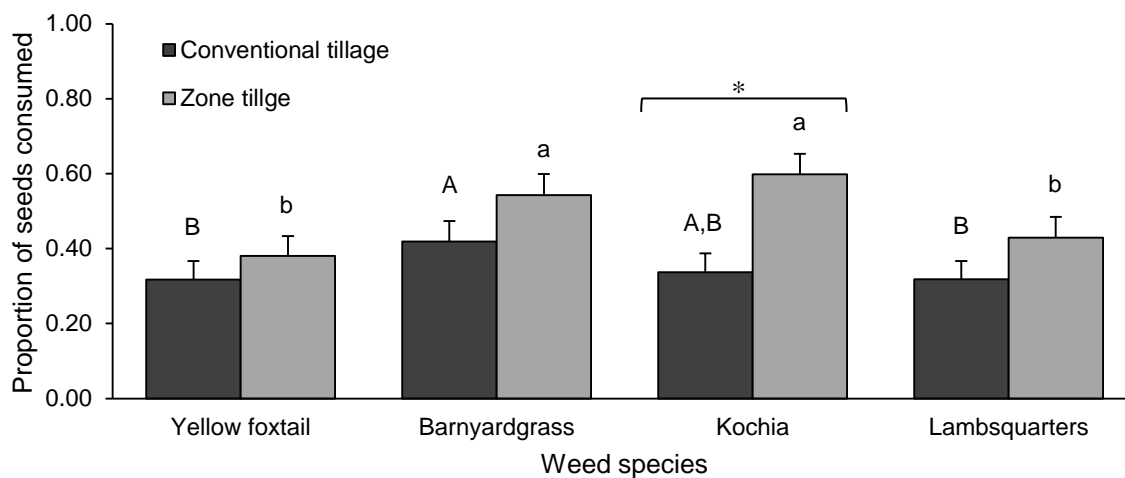
\* = Significant differences between the two tillage practices within a date (at  $\alpha = 0.05$ ).

**Figure 5.1:** Mean ( $\pm$  SEM) number of ground beetles (a and b), centipedes (c and d), spiders (e and f), and rove beetles (g and h) collected during the 2012 (left-hand figures) and 2013 (right-hand figures) cropping seasons in sugar beets produced by means of two different cultivation practices (zone tillage and conventional tillage). Sample points on the x-axis indicate the date on which samples were collected from the field. Data analyzed by means of two-way ANOVA with repeated measures.

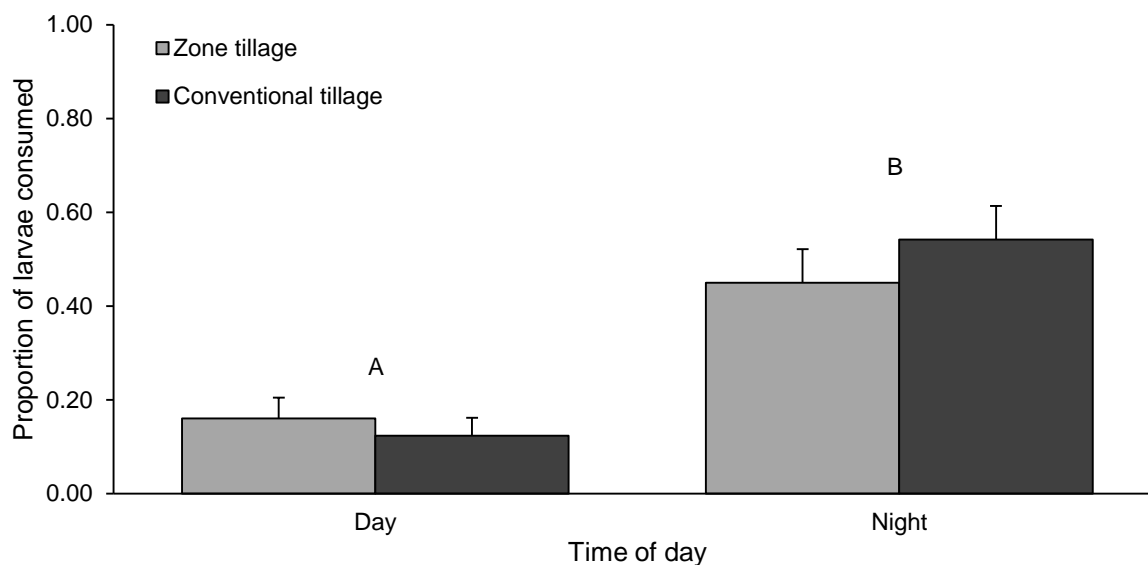




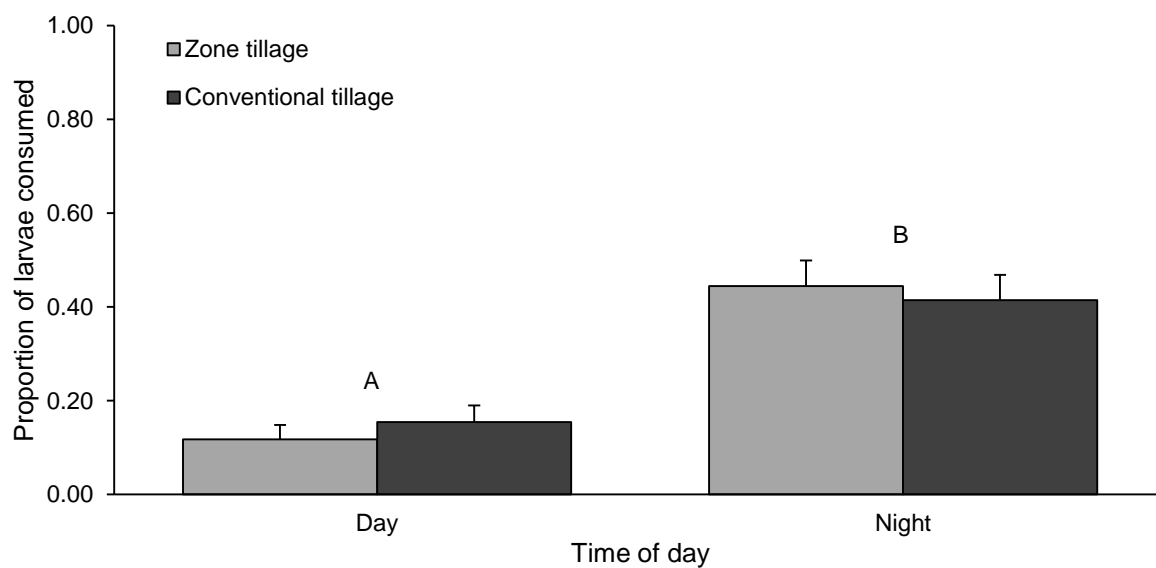
**Figure 5.2:** Mean proportion ( $\pm$  SEM) of weed seeds consumed during the 2012 field season for four different weed species in conventional- and zone tillage plots by beneficial arthropods. Weed species with different letters differed significantly in the rate of their consumption by beneficial arthropods (LSD test,  $\alpha = 0.05$ ).



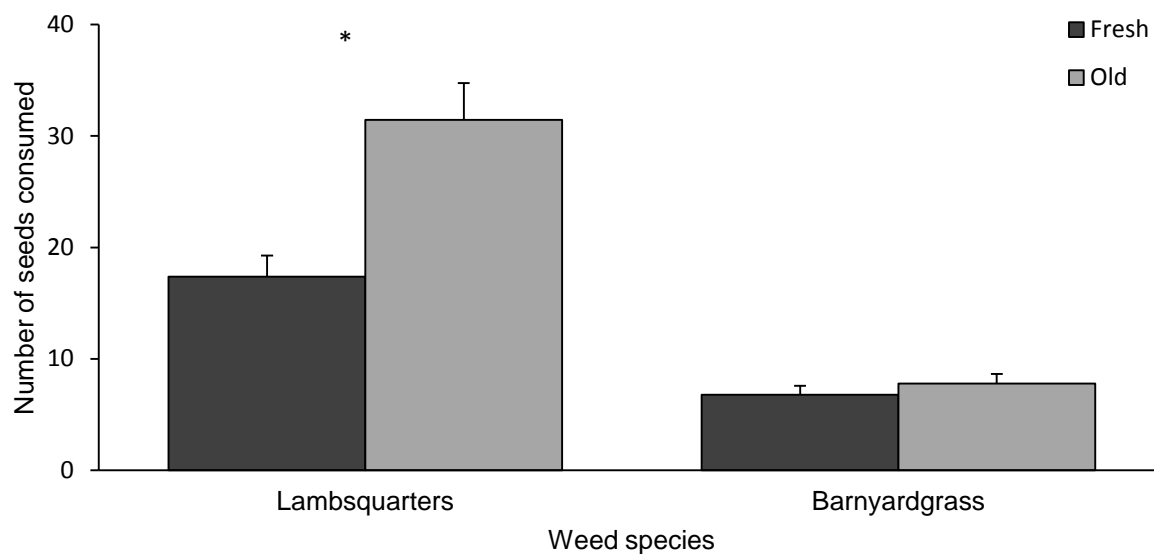
**Figure 5.3:** Mean proportion ( $\pm$  SEM) of weed seeds consumed during the 2013 field season for four different weed species in conventional- and zone tillage plots by beneficial arthropods. Different letters indicate significant differences between weed species within a particular tillage system (capitalized letters = conventional tillage; lower case letters = zone tillage). Weed species with an asterisk indicates significant differences between tillage practices (LSD test,  $\alpha = 0.05$ ).



**Figure 5.4:** Mean proportion ( $\pm$  SEM) of waxworm larvae (*G. mellonella*) consumed during the 2012 field season in the conventional- and zone tillage plots during different times of the day (day: 07:00 am – 18:00 pm; night: 19:00 pm – 06:00 am). Time periods with different letters are significantly different (LSD test,  $\alpha = 0.05$ ).



**Figure 5.5:** Mean proportion ( $\pm$  SEM) of waxworm larvae (*G. mellonella*) consumed during the 2013 field season in the conventional- and zone tillage plots during different times of the day (day: 07:00 am – 18:00 pm; night: 19:00 pm – 06:00 am). Time periods with different letters are significantly different (LSD test,  $\alpha = 0.05$ ).



**Figure 5.6:** Mean number ( $\pm$  SEM) fresh and old barnyardgrass and lambsquarters seeds consumed by *Harpalus pensylvanicus* over a 30 h period. Bars with an asterisk indicate a significant difference in the number of seeds consumed between the seed age groups (LSD test,  $\alpha = 0.05$ ).

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